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THE AMERICAN
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THE COMBINED EFFECTS OF CONVULSANT AGENTS AND LIGATION OF THE HEAD ARTERIES IN CATS

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Muskens (1928, pp. 4 and 5) mentions experiments by Hill (1910) to the effect that when the four cerebral arteries were compressed in cats or monkeys there were no convulsions when absinth was injected. Stewart and Pike are said to have corroborated this. Hill makes no statement concerning absinth in the place cited and Stewart and Pike did not use absinth in any of their experiments. It seemed desirable to ascertain what actually does happen when the head arteries of cats are occluded temporarily at varying intervals before or after the intravenous injection of doses of absinth or camphor monobromide known to be above the minimal convulsive level.

EXPERIMENTAL PROCEDURES. Cats were used in all the experiments. The head arteries were exposed under ether anesthesia in the manner described by Stewart and others (1906). The femoral artery and vein of one side were exposed and the artery used for blood pressure. Respiratory movements were registered by a Crile stethograph placed at the level of the diaphragm, and a recording tambour. After taking a control record of respiration and blood pressure, the operation wounds were flooded with novocaine and ether was intermitted. Two main procedures were followed in the experiments.

I. Absinth or camphor monobromide was injected into the femoral vein and the head arteries were occluded at an interval varying from twenty seconds to two minutes afterward.

II. Occlusion of the head arteries for varying periods of time followed at varying intervals by injection of the convulsant drug.

I. When the animals showed a good return of cerebral function following the control occlusion of about three minutes, and the subsequent release of the head arteries, the convulsant drug was injected. The head arteries

were again occluded in order to observe the effect of the combined procedures.

Control observations have shown that a vigorous animal will withstand fourteen to eighteen successive occlusions of the head arteries with periods of restoration of the cerebral circulation intervening. Animals will also withstand from ten to twelve successive injections of the convulsant drug if the dose is kept below the lethal point.

When the occlusion of the head arteries is done about twenty seconds after the injection of the absinth, before the convulsions appear, or have become well established, the gravity and duration of the effect due to the drug are much reduced, and sometimes no convulsions appear at all. But after the release of the head arteries, and the return of cerebral function, the drug effect may appear with practically the same severity which it would have manifested following the injection if the arteries had not been occluded, provided the period of occlusion has not exceeded three to five minutes. When the period of occlusion is longer (six to ten minutes) no generalized convulsion occurs in the recovery period, the most marked effect being the appearance of single twitches, generally not involving all the limbs at the same time. The animal succumbs after five or six repetitions of the combined procedure—the injection of the drug followed by occlusions of the head arteries. Blood pressure rises higher during the early occlusions following the injection of the drugs than it did during the control occlusions. The vasomotor response diminishes thereafter with each successive occlusion and finally fails completely.

When an interval of thirty to sixty seconds elapses between the injection of the drug and the occlusion of the head arteries, the convulsive effects due to the drug usually become well established but cease sooner after the occlusion than they otherwise would. No sustained generalized convulsions were observed in the period following restoration of the circulation to the head. The rise of blood pressure during the occlusion was, in general, noticeably greater than that during the control occlusion. The graphic records show that the cardio-vascular response due to the occlusion is superposed upon the rise of blood pressure accompanying the clonic movements of the muscles during the convulsion due to the drug.

When longer intervals (one to two minutes) intervene between the injection of the drug and the occlusion of the head arteries, no convulsive movements due to the drug or to the occlusion itself occur in the period of recovery after release of the head arteries. Respiration usually returns rather early, but the return of the corneal reflex is often delayed ten minutes or more. Blood pressure during the occlusion sometimes rises almost as much as in the previous observations, but the duration of this anemic rise is less than in the control period. Animals usually cannot withstand more than two such combined procedures. Histological examination of the

region of the cortex around the cruciate fissure, stained by Nissl's method, shows chromatolysis, a feebly-stained, swollen nucleus and a shrunken appearance of the cell bodies, with prominent processes.   The processes appear more prominent under these conditions than in the case of animals which have had drugs or have been subjected to anemia only.

As the experiment proceeds, the clonic type of response due to the drug, which appears in the early occlusions, becomes less marked, while the tonic element becomes more noticeable until, at the close, it is the only type of response to be observed.

In three of the experiments a peculiar double effect—that is, a dissociation of the cardio-vascular response following occlusion into two parts—a rise and fall and a second rise and fall—was observed.   This has been seen in the control cats after twelve to fifteen successive occlusions of the head arteries (Winkin, 1922).   At post mortem the stomach appeared much dilated and there were the usual petechial hemorrhages in the lungs.   The appearance of rigor mortis was delayed.

II. The procedure was now reversed.   The head arteries were occluded for from three to fifteen minutes, after which the circulation to the head was reëstablished.   At varying intervals in the period of recovery, the convulsant drug was injected.

Where the duration of the occlusion of the head arteries was from three to five minutes, a quantity of the drug equivalent to the minimal convulsive dose under control conditions would elicit a typical drug convulsion within ten to fifteen minutes after restoration of the cerebral circulation.   Several repetitions were possible, although the number of combined procedures was markedly less than in the case of either one alone.

If the occlusion period was from five to nine minutes, the interval elapsing between the time of reëstablishment of the cerebral circulation and the time at which the injection of the convulsant drug became effective, was increased to thirty or forty minutes.   Injection of the drug earlier than this was generally followed by a preliminary fall and then a rise of blood pressure, sometimes of considerable extent.   With the passing off of this blood pressure response, or even during the response itself, respiratory movements were diminished or even suspended for twenty to thirty seconds.

When the longer interval elapsed before the injection of the drug, the convulsion was neither so long nor so severe as under control conditions, with a considerable emphasis on the tonic element.   The animals sometimes succumbed after the first injection of absinth and few survived the second injection.

Where the period of occlusion was from nine to fifteen minutes, injection of the convulsant drug became effective only after the lapse of forty-five to sixty minutes following restoration of the circulation to the head, and sometimes never was effective.   Frequently tonic movements alone were elicited

by the drug, although respiration and blood pressure might be well established and the corneal reflex present. The procedure could not be repeated, since blood pressure fell to spinal level during the second period of anemia and could never be restored. No asphyxial struggles were ever present.

Post-mortem examination usually showed dilatation of the stomach and petechial hemorrhages in the lungs. The appearance of rigor mortis was usually delayed beyond an hour.

GENERAL COMMENT ON THE RESULTS. The results reported in this paper enable us to bring together certain facts on the action of the convulsive agents observed under other conditions. We have shown previously that the rise of blood pressure following intravenous injection of a convulsant agent is due to the action of the skeletal musculature during the period of clonic convulsions. It has been shown also that the rise of blood pressure following occlusion of the head arteries is due to vaso-constriction. The greater magnitude of the total rise of blood pressure following occlusion of the head arteries at the height of the rise due to the clonic movements after the injection of a drug becomes understandable. The drug itself has not produced any vaso-constriction. The vaso-constriction due to the occlusion of the head arteries produces a further rise of blood pressure superposed on that originating from the action of the skeletal musculature, sometimes greater than that following either procedure alone.

The briefer duration of life in an animal subjected to both experimental procedures, i.e., a convulsant agent and anemia, also becomes understandable. Either procedure alone leads to injury of the ganglion cells of the central nervous system as has been shown histologically. The combined effects of the two procedures would be simply a summation of the effect of each taken separately. The failure of the clonic response to the convulsant agent before the disappearance of the tonic movements falls in line with the known damage occurring about the cruciate area. These results are in agreement with those observed after an anatomical lesion about the cruciate fissure. They become, therefore, a part of the general argument on the localization within the central nervous system of the cells from which convulsive phenomena arise (Pike, and others, 1930, 1931).

SUMMARY

1. Convulsions induced by intravenous injection of camphor monobromide or absinth may be stopped by occlusion of the arteries to the head, if this be done within 20 to 50 seconds after the injection of the drug.

2. If the convulsion is arrested by occlusion of the head arteries, before it is well under way, it will reappear after resuscitation of the animal, provided the period of anemia inflicted has not been longer than 4 to 7 minutes.

3. The type of convulsive movements, tonic or clonic, which appear on

injection of the convulsant agent following periods of anemia is related to the length of the occlusion period.

4. If the occlusion of the head arteries is done at the time when blood pressure has risen during the clonic convulsion, the anemic rise is added to the convulsive and appears to be the maximal blood pressure of which the animal is capable.

5. Without the active participation of the skeletal musculature, neither the convulsive, nor the anemic rise of blood pressure, under these conditions, is significant.

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MECHANISM OF THE POSTURAL REDUCTION IN VITAL CAPACITY IN RELATION TO ORTHOPNEA AND STORAGE OF BLOOD IN THE LUNGS

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It has been known for eighty years that a change from the upright posture reduces the vital capacity of normal individuals (1, 2, 3, 4, 5, 6, 15). The mechanism of this response is not at all clear and the relation which it bears to orthopnea seems quite a mystery.

Mechanical impediments to respiration had early been postulated. Lauder Brunton (7) suggested that since the diaphragm has to raise and lower the viscera when one is recumbent but merely to move them horizontally when one is upright, we have here a key to the limitations which posture places upon breathing. Other authors have spoken of a supposed difficulty of fixing the shoulders and working the auxiliary respiratory muscles when one is recumbent.

The importance of mechanical factors such as these is rendered doubtful by the observation of Peabody et al. (8) that weakness or fatigue has little or no effect of lowering the vital capacity, and the well known fact that orthopnea is not related to general muscular weakness.

Several authors have associated reduced vital capacity in heart disease with the circulatory apparatus. The reduction may be due to the congested alveolar walls (9, 10). The augmented venous return in recumbency can perhaps be expected to increase the load on the heart and the congestion in the lungs (11). The congestion might reduce the vital capacity, but that it alone could increase the breathing (orthopnea, cardiac dyspnea, 12) seems quite impossible. The dyspnea must be due to increased discharges from the respiratory center, which in turn might be reflex or be due to the fact that the postural change brings in its wake deficient aeration of the blood (13) or deficient circulation through the medulla.

The careful study of many factors associated with the postural reduction of the vital capacity of normal individuals may throw light upon these perplexing questions. This possibility has led us to measure the vital capacity, the residual air and the size of the chest while the subject was standing

and again while he was lying down. Further important information was obtained from the subject lying supine on a ⊥ shaped scaffold so arranged that the body weight was supported by the hips on the cross bar and by the spines of the dosal vertebrae on the upright. Our tentative conclusions were substantiated by the effect upon the vital capacity of compressing the bases of the four extremities.

A. *The vital capacity* was measured standing, lying and on the scaffold. The series comprises only five individuals but each result represents the average of many determinations.

Since the postural reduction in vital capacity is almost as great when one rests on his spinous processes and hips as when one rests on his posterior chest wall, we have concluded that the breathing is but little embarrassed by the weight of the body on the ribs.

The residual air was calculated as described by Hamilton, Moore and Kinsman (14) from the $O_2 + CO_2$ after ventilating the lungs with O_2,

TABLE 1

Vital capacities in different postures

SUBJECT	STANDING	LYING ON ⊥	LYING SUPINE
C. M.	4150		3987
A. B. M.	6000	5580	5580
W. F. H.	4420	4175	4235
J. G.	3765	3670	3530
R. M.	3400	3140	2940
Average	4347		4054

exhaling to the residual air, and rebreathing a known quantity of nitrogen to a complete mixture. The results are given below. Each figure is the average of six to ten determinations.

There seems to be no significant difference between the residual air in the two postures. This is rather surprising in view of the results of Bohr (4) and Plesch (15) whose work is usually taken to indicate that the residual air increases when the subject lies down. Bohr did not determine the residual air directly. It was left to be determined by subtracting the reserve air from the mid-capacity. It is interesting also to note that the mid-capacity and complemental air in the various postures always add up to an identity, which figure equals the total capacity as determined in the preliminary measurements (standing?). We are, therefore, left with the suspicion that the mid-capacity was not determined separately in each experiment but that the figure published was merely the result of subtracting the complemental air from the previously determined total capacity. We are not, therefore, justified in drawing conclusions (16)

as to the effect of posture upon the residual air from Bohr's data unless we accept the rather gratuitous assumption that posture has no effect upon the total capacity.   The residual air figures of Plesch (15) bear the appearance of having been arrived at indirectly and in somewhat the same fashion.

Our measurements of the residual air and vital capacity indicate that the total capacity which is the sum of the two, is reduced when one lies down.   If this is true, one might expect that the chest size would be different either in the expired or inspired position or in both, depending upon whether the subject were lying down or standing up.

TABLE 2

Residual air in different postures

SUBJECT	STANDING	LYING ON ⊥	SUPINE
C. M.	808		842
A. B. M.	1062	1107	1085
W. F. H.	936	835	907
J. G.	969	820	763
R. M.	772		765
Average	909		907

TABLE 3

Comparison of vital capacity (average of four to eight determinations) with and without mechanical congestion of extremities (cuffs)

	WITHOUT CUFFS		WITH CUFFS	
	Recumbent	Standing	Recumbent	Standing
W. F. H.	4275	4614	4583	4644
A. B. M.	5880	6233	6333	6350
W.	3275	3480	3650	3510
K.	4319	4650	4454	4643

The circumference of the chest in two of our subjects appears by measurement slightly larger when the subject is lying down.   This is true in both complete inspiration and complete expiration.   The significance of this appearance is doubtful because the tape may have been pulled about by the subject's flesh in contact with the table.

To eliminate this difficulty, a set of calipers was made to determine the antero-posterior diameter of the chest from the third sternocostal junction in front to the fifth dorsal spine behind.   The measurements, taken at full inspiration and full expiration, on three subjects showed that the chest was from 2 to 10 mm. thicker when the subject was recumbent.

The width of the chest was measured at the level of the greatest excursion of the sixth rib on x-ray plates. The plates were taken with the tube six feet away from a mark on the chest and in a direction from this mark perpendicular to the surface against which the subject was leaning or upon which he was lying. The measurements showed when the subject was recumbent that in the ten comparisons (five subjects) the chest was wider by 2 to 10 mm. in all but two comparisons. In these two (full inspiration) the width of the chest was the same in the two postures.

The height of the diaphragm is more variable. In half the cases it was higher in the recumbent position and in half it was lower. The differences in height, ranging from 2 to 15 mm., indicate that on the average the height of the diaphragm in our subjects is about the same in the two postures, when the measurements are taken at full inspiration and full expiration.

The chest then may be taken as somewhat larger when one lies down. This applies only to measurements taken at complete inspiration and complete expiration. The residual air, however, is the same in the two postures and the total capacity is less lying down even though the chest is no smaller or sometimes even larger in this posture.

This raises the question: What may occupy space in the recumbent chest when the residual air is the same and the expired chest size increased; or when the total capacity is decreased with an increased or at least unchanged chest size?

The most convenient answer is that there is a greater amount of blood in the chest when the subject is recumbent. To test this hypothesis blood pressure cuffs (17, 18) were fitted around the bases of both arms and both legs. The vital capacity was taken standing and recumbent. The blood pressure cuffs were inflated to diastolic pressure and left for five minutes. The vital capacity measurements were then repeated and showed a marked increase in the vital capacity in the recumbent position. The increase in the upright position is not so great.

(It was found further that in certain cases of cardiac disease the vital capacity was increased by the application of the cuffs.)

This indicates strongly that the pulmonary congestion of recumbency is to a great extent responsible for the reduced vital capacity of recumbency.

The mechanism governing the phenomena which are glimpsed from the standpoint of these experiments is undoubtedly very intricate. How, for instance, is the residual air kept at about the same volume even though the chest size is not? A mechanical end-point may play a part, since the face and neck are markedly engorged when one exhales to the residual air in the recumbent position. This engorgement is not very noticeable when the subject is standing or when the cuffs are applied to the extremities (recumbent). To assume, however, that the expiratory stop is produced

mechanically within the lung is to assume that the intrapleural pressure is positive on expiration, in the recumbent position and that the residual air equals the minimal air. Both these assumptions seem unlikely. Further, if a mechanical end-point were responsible in stopping the expiration, one might expect the rigidity of the engorged lungs to stop expiration when the residual air was above the normal value.

On the contrary, it is very interesting that expiration stops at practically the same residual figure, in both postures and even when the vital capacity is markedly reduced in cardiac disease (congestive failure) (19, 20) (cf. 21). This fact indicates the possibility, at least, that a reflex mechanism similar to that described by Hering and Breuer (22) sets up impulses which inhibit the most active voluntary expiratory movements before the alveoli become so collapsed as to lead to their injury.

The fact that the lungs may be used to store blood is a matter of some significance. The hydrostatic increase in venous pressure at the heart (23) which occurs upon lying down is, we think, an important link in the chain of factors which causes the lungs to increase their blood content during recumbency.[1]

This increase in venous pressure is due to the fact that when one lies down, the blood which has been stagnating in the dependent veins is poured toward the heart. It is at once pumped into the lungs. Since all of the known reflexes which regulate the rate of the heart have their receptors on parts of the systemic circulation-path, we are justified in saying that the rate at which blood is pumped out of the heart seems to be regulated by the *systemic* demand. Thus the recumbent subject, resting, will have a relatively narrow systemic vascular bed and the heart rate will be slowed down reflexly, keeping the arterial pressure at about the usual level. Blood which had in the upright position been stored in the veins, is now stored in the lungs.

The stroke volume of the recumbent subject may be assumed to increase. This follows whether one accepts the cardiac ouput figures of Grollman (16) or those of the earlier workers (26, 27, 28). On the right side of the heart this increased stroke volume is to be looked upon as a result of the increased effective venous pressure in the great systemic veins. If we assume the venous pressures in the standing subject to be well below the

[1] It must be remembered, as was pointed out by Henderson and Haggard in 1918 (23), that when venous pressure determinations are made from the veins in the arm or hand, they bear no relation to the pressure at the portals of the heart when the readings are taken with the subject in the upright posture. They are expressions merely of the height of the observed vein in relation to the height of the axillary vein, through which the blood must be forced on its way to the heart. Neglect to properly evaluate this simple example of hydrostatics is responsible for the fact that we have statements in the literature that venous pressure decreases on lying down and even find this "fact" referred to (26) as "unknown compensatory mechanisms!"

"critical" level as indicated by the low stroke volume, there must be a similar or even greater (29) increase in pressure at the portals of the left heart in order to fill the left ventricle to correspond with the filling of the right ventricle. This pressure in order to be effective must be communicated by the right heart through the capillary bed of the lungs and to the pulmonary veins.

The vascular bed of the lungs can be regarded as easily subject to distention as pressure increases (10, 30). This fact gives the lungs the function of storing blood though, as we have seen, at the expense of their respiratory function.

This store of arterialized blood is increased and made manifest in recumbency though it probably exists at all times. Its value in sudden emergency is, of course, a matter for speculation, being comparable to that of the spleen.

Barcroft and Stevens, in their analysis of spleen function, have described graphically the possible rôle of stored blood in the circulatory economy, which may be paraphrased as follows:

Assuming a total blood volume of five liters, and that the spleen can discharge one liter of blood in twelve seconds, if his minute volume were five liters (i.e., one liter in twelve seconds) the splenic function would double the cardiac inflow and hence output. Assuming now a readjustment of vascular conditions to continue this doubled inflow during the emergency, the circulation rate will have become doubled until something takes place to alter that condition of affairs.

It is quite possible that blood stored in the lungs may play a rôle, similar to that of splenic blood, in times of emergency.

To make use of this reserve blood, the vessels of the muscles have merely to dilate (exercise) and the blood will be pumped out of the lungs by the left heart.

The blood storage function of the lungs encroaches, as we have seen even in the normal individual, upon their respiratory function. If the left heart is weakened as in ordinary forms of heart disease, the organism suffers from failure of the left heart to pump blood from the lungs until the pressure in the pulmonary system is markedly increased and the storage of blood in the lungs has encroached severely upon their respiratory function. These facts are evidenced by the reduced vital capacity and the signs of pulmonary congestion which accompany this condition. Matters are now made worse by assuming the recumbent position. The blood storage capacity is still more over-strained, aeration of the blood becomes less efficient and the symptoms of orthopnea supervene.

SUMMARY

1. Evidence is presented that while the vital capacity decreases on assuming the recumbent posture, the residual air remains constant.

2. In the completely inspired position the size of the chest may increase in the recumbent posture.

3. In the completely expired position the size of the chest usually increases in the recumbent posture.

4. It would seem, from the above, that the lungs serve as storage for blood which is poured out of the dependent veins when one lies down.

5. This conception is substantiated by the fact that when blood pressure cuffs are applied to the four extremities, and blood trapped in the arms and legs by raising the pressure in the cuffs to diastolic, there is a prompt and marked increase in the recumbent vital capacity, so that it may equal or exceed the vital capacity in the standing posture.

The mechanism of blood storage in the lungs is discussed. Its rôle in an emergency is suggested and its relation to orthopnea is pointed out.

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STUDIES ON THE CIRCULATION

IV. FURTHER ANALYSIS OF THE INJECTION METHOD, AND OF CHANGES IN HEMODYNAMICS UNDER PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

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As the injection method has developed (1, 2, 3, 4, 7), several questions have arisen in our minds, as well as in the minds of those who have constructively criticised it (15). These questions involve consideration of the mathematical basis of the time-concentration curve of consecutive arterial samples, when a dye is injected into a vein; the calculation of the volume of blood in the thorax; and the effect upon the curve of such factors as different rates of flow in parallel streams (as might occur in the lungs), of mixing of dye in the heart and great vessels, and of diffusibility of various dyes.

The purpose of this paper is to describe in detail the present technique of the procedure; to present new evidence in answer to the above questions, and to illustrate the application of the method with data gathered from experiments on man and animals.

Technical details of experimental procedures and calculations. In the experiments on animals and with artificial systems, the technique is the same as that already described (1, 2, 3, 4). In the experiments on man, the puncture technique has been modified. We have attempted (1, 3, 4), with varying success, punctures of the radial, brachial and femoral arteries. The one most easily punctured is the femoral but has necessitated an awkward and uncomfortable posture on the part of the subject. This difficulty has been eliminated by having the subject near the edge of the bed with a semicircular piece (to receive the sampling kymograph) about a foot in radius removed from the center of the edge of the mattress and of a wooden platform under the mattress and on the bed. He is supported with pillows in a comfortable position which is halfway between the supine and lateral-recumbent posture. A needle, which has been found satisfactory for sampling from the femoral artery, is made from 19 G. stainless steel needle tubing. Two centimeters from the sharpened end is a 45° bend. One centimeter behind this, a collar is sweated on to facilitate handling. Three or 4 cm. behind this and at the other end of the needle is a 90° bend in the same direction as the 45° bend. A needle of this shape cannot be cleaned with a stylet;

flushing with two per cent alkali, however, keeps it clear. If the puncture sites are properly novocainized and the work carried out calmly and surely, the samples can all be taken very quickly, with little excitement or anxiety on the part of the subject, as evidenced by lack of increase in heart rate.

The concentration of the dye in the samples is determined colorimetrically in a micro-colorimeter (B and L) against a standard made up to known strength.

The dilutions are made in essentially the same fashion as before described (1), except that with brilliant vital red which we are now using,[1] 0.01 per cent NaOH solution is substituted for the 1 per cent NaOH which was used with phenoltetraiodophthalein.

It occasionally happens that when the two standards are read in the colorimeter against each other, the weaker standard gives a higher reading than it should. This is because of adventitious color which can be recognized in the undyed serum, as serum pigment or dye left over from a previous experiment. This adventitious color cannot be read against the blood standard because the latter is sophisticated by the same color. It is the same in both standards but bears in each a different relation to the added dye. Its amount can, therefore, be calculated by

$$\frac{x + 125}{x + 250} = \frac{R\ 250}{R\ 125} \qquad\qquad \text{I}$$

where x is the unknown adventitious color, 125 and 250 are the concentrations in milligrams per 1. of dye added to the blood for the weak and strong standards respectively, and $R\ 125$ and $R\ 250$ are the actual readings at which the weak and strong standards match. Thus, if the readings are $R\ 250 = 4$ and $R\ 125 = 7.1$ the adventitious color would come out as equal to a dye solution whose concentration is 25 mgm. per 1. in blood. The standards would then be considered as containing 150 and 275 mgm. per 1. For convenience in calculation the standards are set at the reciprocals of these figures, viz., 6.67 mm. and 3.33 mm. or a multiple, and the samples read as indicated in the previous section. The figure representing the adventitious color would, of course, have to be subtracted from the value attached to each sample.

In practice one usually finds that the two standards give a good match at depths which correspond to their supposed concentrations. The equation then would work out $x = 0$ and no allowance would have to be made for adventitious color. An occasional sample, however, shows a color which is of a different hue in the colorimeter, and whose best match gives a reading that diverges widely from that of the rest of the series. Such a sample has been exposed to some accident that has caused hemolysis and should be disregarded.

After the readings are made, and corrected if necessary, they are plotted as ordinatés on semi-logarithmic paper (Keuffel & Esser Co., N. Y., no. 358-70) against time in seconds as determined by measuring from the middle of the injection mark to the middle of each tube. A smooth curve is then drawn through the points on the upstroke and peak of the curve. The points on the downstroke will, if the work has been properly done, indicate a straight line. This line is then drawn through these points and prolonged through one cycle of the paper (e.g., from ordinate 100 to ordinate 10).

After the curve is drawn as described above, the next step is to read and add together (S_1) the height, in milligrams per liter, of the intersection of every second-ordinate on the curve, from its beginning to where, on the downstroke, it enters the

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[1] Congo Red, Niagara Sky Blue 6 B, and Special Blue T. S. S. (National Aniline Co.) were all used and found to be less satisfactory than the Brilliant Vital Red.

straight line. The ordinate values of the points at second intervals on the straight line as it crosses the first cycle on its downward course are summated separately (S_2). As this line crosses the next cycle (10 to 1) with unchanging slope, the summed points (S_3) will be, very closely, one-tenth of the summed points in the cycle above (S_2). The next cycle will contribute (S_4) one-hundredth of the value of S_2 and so on. The sum (S) of all the values, in mgm./l. read at each second's intersection of the curve, when the curve is prolonged to infinity would be $S_1 + S_2 + S_3 + S_4$, etc. How far it is necessary for practical purposes to prolong the series depends upon its slope; the larger S_2, S_3, S_4, etc., are, the more nearly horizontal the curve. One's judgement, and the accuracy required of the calculation, should easily determine the end point.

For convenience in calculating, the formula (2, 3)

$$F = \frac{60\ i}{c\ t} \qquad\qquad \text{II}$$

has been simplified to

$$F = \frac{60\ i}{s} \qquad\qquad \text{III}$$

where F is the flow in liters per minute, c is the average concentration in mgm./l. during the primary curve, and t is the duration in seconds of the curve used in the calculation. Since $ct = \frac{s}{l}\ t$ the arithmetic is simpler with the second equation.

VOLUME-FLOW-CONCENTRATION RELATIONSHIPS IN SIMPLE SYSTEMS. When dye is injected into the vein of an experimental animal and consecutive samples taken from some point in the arterial system, it is seen that the curve of concentration rises to a maximum, rounds off, and descends a little more slowly than it ascended; and that there is unmistakable evidence (2, 3) that the logarithm of the concentration finally assumes a linear relationship with time. The relationship found in the descending limb of the curve is expressed by the compound interest law (2) (for details of which any text on calculus can be consulted). The formula is as follows:

$$C_t = C_o\ e^{-\frac{f}{XV}t} \qquad\qquad \text{IV}$$

Where C_o is the original concentration in milligram per liter.

C_t is the concentration after the elapse of t seconds.

e is the mathematical constant 2.718+.

f is the flow in liters per second.

V is the volume of the system in liters.

X is a factor to correct for the fact that mixing is not instantaneous.

This equation cannot be employed in calculating quantitative data on account of the difficulty of assigning a definite value to X. It is introduced here for the purpose of emphasizing the fact that blood flows through the lungs in a manner that is essentially predictable, and washes

out the injected substance in such a fashion that the time-concentration relations conform to a mathematical formula. X can be determined by substituting for V in equation IV, its value as obtained by multiplying the mean circulation time by the flow (*vide infra*), and would be an index as to how large a part of the volume between the needles the dye was simultaneously mixed with. The physiological significance of this factor is at present undetermined.

Although from the evidence published above and that cited elsewhere it seems clear that one may calculate the flow from the time-concentration relationships, it has not been demonstrated conclusively that the volume of the system carrying the flow can be calculated with equal assurance. Indications of the nature of this demonstration have already been published (2).

It was assumed by Stewart (5) and the assumption accepted by Blumgart and Weiss (6), that the fastest circulation time does not differ materially from the mean circulation time. This idea rests upon the argument that in flowing through a complicated capillary pathway a particle of blood is passed in and out of the axial stream so many times that its average velocity would in the end be no different from that of all other particles in the same stream. The argument neglects the obvious fact that some pathways are longer and more tortuous than others, and hence take more time in passage. A casual glance at the curves given in this and earlier papers, particularly the heart-lung perfusion curves, leads inevitably to the conclusion that the shortest circulation time is *not* of the same order as the mean or average circulation time.

We at first thought it possible to determine the average time by finding the ordinate which divided the area of the curve into two equal parts (2). If the curve is symmetrical this procedure is adequate, and most of the curves with which we first checked the volume calculation were nearly symmetrical, and the calculations checked. They failed to check in curves from the more complicated systems. These curves were markedly asymmetrical and so were those from the cases of decompensated heart disease.

The average time M it takes the dye to go through a system can be calculated from the time-concentration curve by the following formula:

$$M = \frac{T_1C_1 + T_2C_2 + \ldots\ldots + T_nC_n}{C_1 + C_2 + \ldots\ldots + C_n} \qquad \text{V}$$

Let the concentration readings be $C_1, C_2, \ldots\ldots C_n$ at times $T_1, T_2, \ldots\ldots$ T_n. M is the T-coördinate of the center of gravity of the curve. The curve may also be replotted on linear coördinates and cut out. The T-coördinate upon which the piece of paper (of uniform thickness) thus formed balances on a knife edge is approximately the T-coördinate of the

center of gravity of the curve, and the time from the middle of the injection period to this time, when multiplied by the flow, gives the volume of the system within the limits of experimental error.

The mean circulation time has its importance from the obvious fact (5, 6), that during this time the flow will have displaced a volume equal to that within the system, and hence that

$$V = M f \qquad\qquad VI$$

where V = the volume contained in the system, M, the average time it takes the injected substance to pass through the system, and f the flow per second.   Using this formula we find that V is correct within the limits of colorimetry in all sorts of artificial models in some of which the dye solutions flow through empty bulbs, in some through bead-filled bulbs and in some through combinations of these in series or in parallel.

VOLUME-FLOW-CONCENTRATION RELATIONSHIPS IN COMPLEX SYSTEMS. If the flow through each of two capillary beds is the same in proportion to their volume the curves of dye concentration in successive samples
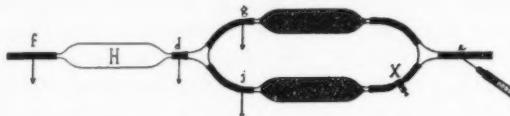
Fig. 1. Apparatus through which dye is carried by a stream of water.   Bulbs in parallel are filled with beads.

taken at the exit of each system are of course identical.   See figure 1.   The mixture of the two identical streams would have the same time-concentration relationships as either one.   Experiments have shown that it is possible to calculate both flow and volume within the limits of experimental error in set-ups of this type.

If, on the other hand, the flow through the two parallel capillary beds is not in the same proportion to their volume, the time-concentration curves assume bizarre shapes, simulating roughly curves in which recirculation has occurred.   Figure 2 has been constructed in such a way that in case of b_3 the flow through one bulb is twice that in the other bulb of the same size (see fig. 1); in case of b_4 it is three times, in b_5, four times and b_6, five times.

The flows and volumes as calculated from the curves using equations III, V and VI, have been found to agree within one or two per cent with the flows determined on the basis of the experiments used in constructing the curves.

Now, if we attempt to get at these quantities (V and F) by prolonging the straight line in the earlier part of the curve—a procedure which is necessitated by the situation in animal experiments—we get the figures given in table 1.

From this it would seem that when an error appears which is due to failure of the two branches of the stream to flow at the same rate in proportion to their volume, there is a limit to the degree to which the flow figures may be affected. The above possibilities which we have tested graphically involve the assumption that the two beds with unequal flow have equal capacity. If, however, the slower bed have the smaller capacity, the error would *ipso facto* be proportionately reduced. If, on the other hand, the slower bed have the larger capacity, an incongruity might arise in that the smaller and faster bed would predominate in the volume calculation and give a result which might warn one that something was awry.

It must not be thought that in the animal body the possibilities which we have analyzed in detail are permitted by the situation to bring about errors of as large magnitude as those which have come out in these illustrations. When dye is injected into a vein it first mixes in the great veins and right heart with a volume of blood (part of V), is then separated

TABLE 1

Comparison of flows and volumes with those calculated from "prolongation" of the curves in figure 2

CURVE	CALCULATED F	CALCULATED		
		Error	V	Error
	cc./min.	per cent	cc.	per cent
A	1,080	0	112.5	+4.6
b₃	1,860	12.4	228.0	+6.0
b₄	1,844	21.7	197.0	−8.4
b₅	1,670	18.2	175.0	−18.6
b₆	1,543	16.2	157.0	−27.0

into many parallel paths and finally combines and is mixed further and with more blood in the left heart and arteries, before it is sampled for the concentration curve.

In order to inquire into the significance of these factors, an experiment was performed in which the flow through a set-up such as figure 1 was sampled for a time-concentration curve. The flow was much faster through one bulb than through the other. When the samples were taken at d curve A B D^2 results.

[2] The irregularity (notch) at the beginning of the curve in figure 3 is accidental. The syringe stuck when the dye injection was partly in. The injection was thus made in two parts giving a curve of greater irregularity than was to have been expected from considerations which have appeared above. Other curves more nearly like those in figure 2 could have been substituted for figure 3, but it was thought better to illustrate the influence of common mixing such as would occur in the heart and great vessels, upon this very bizarre curve. The fact that this curve is changed to one that is of the type that is usable physiologically gives a favorable answer to a rather severe test.

If, however, the stream is allowed to pass through a common mixing chamber H, figure 1, and the samples taken at f, figure 1, the curve changes to $A'B'C'$.   These two curves were taken in two rows of sampling tubes on the same kymograph, with one injection of dye.   The flow and volume calculations were as shown in table 2.

Since curve $A\ B\ D$, figure 3, would be hidden by recirculation in a physiological experiment, and hence would not be available, comparison must be made between the figures in the last two columns.   Obviously the mixing in the common parts of the path has done much to eliminate the irregularities in the curve and to facilitate the calculations of both flow and volume.

In animal experiments this factor is important.   Dye in passing through common parts of the path (the heart, great veins and arteries) would mix with the blood in such a fashion as to straighten out irregularities in the
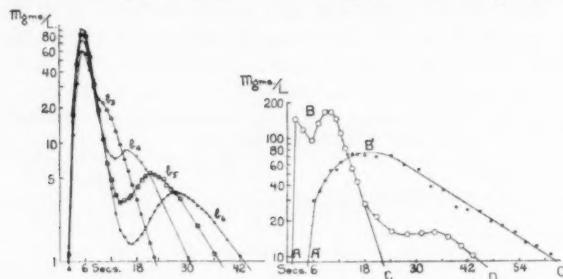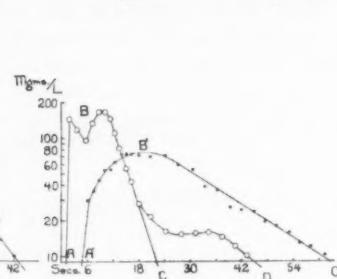
Fig. 2   Fig. 3

Fig. 2. Concentration curves when flow and volume have different relations in two parallel systems.

Fig. 3. The effect of mixing on the concentration curve. ABD samples taken from d, figure 1; $A'B'C'$ samples taken from f, figure 1.

curve which would be impressed by volume flow discrepancies that conceivably might (though they probably do not) occur in the lungs.

In animal experiments it is impossible to determine by inspection of the first circulation curve whether the curve follows the simple single system type or the more complicated type illustrated in figure 2, because it is impossible to determine whether there is a bend in the curve below the point (1, 2, 3) where the concentration begins to increase because of the recirculation of the dye.

Since, however, the injection method gives output figures which check very closely with direct Fick output figures (3) and since these injection figures are based on the assumption that the line does continue straight, the inference is that this assumption is correct.

In order to satisfy ourselves that the ± 10 per cent variation inherent in the results of both methods has not hidden some small systematic error

in the injection procedure, we decided to submit it to further checks (7) with heart-lung perfusion experiments.

HEART-LUNG PERFUSION EXPERIMENTS. *Statement of the problems. The questions that arose* in our minds were as follows:

1. Do the mathematical relationships, which we have referred to above as giving rise to the "straight line," hold when the dye is injected into blood which is perfused through the lungs of an animal?

2. Does dye diffuse through the walls of the lung capillaries in sufficient amounts to distort the relationship between the curve and the true flow?

3. Do these mathematical relationships hold when the lung and left heart alone are perfused without the additional margin of safety furnished by the further mixing which might be expected to occur in the great veins, right heart and arteries?

TABLE 2

Comparison of measured flows and volumes with those calculated from figure 3

	MEASURED	CALCULATED FROM CURVE A B D	CALCULATED FROM CURVE A B C	CALCULATED FROM CURVE A' B' C'
Flow:				
Cc./min................................	990	1,011	1,245	976
Error, per cent...........................		+2.2	+25.7	−1.4
Volume:				
Without H..............................	215	235	153	
With H.................................	470			480
Error, per cent...........................		+9.3	−28.8	+2.0

4. Do they hold whether the flow is slow in relation to the volume, as in human heart disease, or whether the flow is rapid in relation to the volume?

5. Is it possible to obtain any evidence that the quantity V, as calculated, is of the right order of magnitude in animal experiments?

The procedure used in the heart-lung perfusion experiments designed to answer these questions was as follows: About one liter of blood was taken from the hearts of two or three large dogs kept for the purpose. This was heparinized, set aside in a well paraffined flask and kept warm. A small dog—three to five kilos—was bled to death and the blood added to that in the flask. The chest of the small dog was opened, a cannula opening toward the lungs was inserted in the pulmonary artery and one opening toward the heart was inserted in the aorta in such a way as to close the coronaries. The first cannula was connected to a perfusion reservoir by a large vaselined tube 10 mm. in diameter. A 50 cc. glass bulb to take the place of the right heart was inserted between the tube and the cannula. The aortic cannula

was connected to a receiving reservoir by means of a similar tube. The capacities of the tubes, cannulae and bulb were measured.

The lungs were then kept inflated to the size of the chest and perfused several times with the heparinized blood, the flow being regulated to that desired in the particular experiment. Less blood was recovered after the first one or two perfusions, than was originally in the reservoir, indicating that the heart and lungs, under the experimental conditions, contained more blood than immediately after the animal was bled to death. Back pressure in the left ventricle was low; there was no evidence that blood escaped through the Thebesian vessels.

After several perfusions, the kymograph was made ready to receive consecutive timed samples (4) from a needle stuck in the rubber tube as near the aortic cannula as possible. Perfusion was then started and the injection made just above the bulb in the perfusion channel. The samples were set aside to settle and the mixed blood in the receiving cylinder sampled. This procedure was repeated, another injection made and another set of samples taken. The blood was perfused twice more and another sample of the mixed blood set aside. The dye remaining in the blood from the first experiment gives a concentration figure which must be subtracted from each individual determination of the second experiment.

The dye used in these first experiments was usually phenoltetraiodophthalein. This shows a deep blue color in alkali, but remains colorless in serum or water. Brilliant Vital Red, on the other hand, shows its full color in serum or water. Advantage can be taken of this fact to compare successive experiments by injecting into the same perfusing blood first phenoltetraiodophthalein and then Brilliant Vital Red. The serum from the first is diluted with weak alkali and from the second with water.

The experiment was therefore repeated getting samples for two more curves using vital red instead of phenoltetraiodophthalein. Vital red samples were also taken from the receiving reservoir after each perfusion and a sample of the mixed blood taken, two perfusions after the last injection.

From the time-concentration relations of the consecutive samples we were able to plot the curve (see fig. 4) of concentration change from which we could calculate the flow (F) and the width of the intrathoracic vascular bed (V). F could be checked by measuring the flow with a stop-watch and cylinder, and some notion of the accuracy of V could be obtained by comparing the concentration of dye in the receiving chamber with that in the sample taken after the dye had mixed with all the blood in the dog, tubes and reservoirs.

Mathematical relationships. As seen from figure 4, the downstroke of the time-concentration curve in blood perfused through a dog's lung pursues the "straight" course assumed in the discussion.

Diffusibility of the dye. As seen from table 3, the results with phenolte-traiodophthalein differ widely from those with vital red. This is probably due to the diffusion of the former into the tissues for the amount of dye

TABLE 3

Comparison of the measured flow of blood perfused through dog's lungs, with the flow calculated from the results of the injection method using 100 mgm. brilliant vital red and 200 mgm. phenoltetraiodophthalein

PHENOLTETRAIODOPHTHALEIN			VITAL RED		
Observed flow	Calculated flow	Difference	Measured flow	Calculated flow	Difference
cc.	*cc.*	*per cent*	*cc.*	*cc.*	*per cent*
120	170	+41.6	948	946	−0.2
237	355	+50.0	1,150	1,092	−5.1
1,118	888	−20.6	1,170	992	−15.2
1,034	952	−7.9	678	621	−8.4
1,325	1,910	+44.1	882	961	+9.0
1,375	1,840	+34.0	600	612	+2.0
690	900	+30.0	882	998	+13.1
662	853	+29.0			
864	1,100	+27.3			
676	733	+8.4			
769	980	+27.3			
Average.................		+23.9	Average.................		−0.7

TABLE 4

Comparison of phenoltetraiodophthalein with brilliant vital red in determining the human cardiac output by the injection method

CASE	PHENOLTE-TRAIODO-PHTHALEIN OUTPUT	BRILLIANT VITAL RED OUTPUT	DIFFERENCE
	L/mm.	*L/mm.*	*per cent*
Normal C. V. system........................	7.17	6.76	+6.0
Normal................................	8.88	7.11	+25.0
Angina pectoris.............................	6.83	6.61	+3.4
Rheumatic aortic regurg. slightly decompensated................................	9.03	7.61	+18.5
Luetic heart compensated...................	4.56	4.40	+3.6
Average...			+11.2

recoverable in the perfusate was usually lower than expected (−33.5 per cent, −25 per cent, −27 per cent, −18.5 per cent, −14 per cent). In some cases all of the dye could be recovered, which we explain as due to complete washing back of the dye into the blood during the latter part of the perfusion.

Since Brilliant Vital Red is much less diffusible than is phenoltetraiodo-phthalein; since it was recovered completely in the perfusate; and since the output figures agree quite closely with the measured rate of perfusion, we are convinced that with the vital red dye the determinations actually do measure the output of the heart.

Table 4 indicates that in man there is less difference between the phenol-tetraiodophthalein results and the vital red results than one would have been led to expect on the basis of the result of the dog experiments.   The error (of the phenoltetraiodophthalein figures) is small enough to have been covered by the inaccuracies of the injection and direct Fick method in the experiments where these have been compared (2, 3).   The larger differences between the figures from the two dyes in the perfusion experi-ments are no doubt due to the fact that the conditions of the experiment increase the permeability of the lung capillaries so as to let out more phenoltetraiodophthalein but not enough to let out the vital red.

The effect of mixing.   Although the phenoltetraiodophthalein curves did not always present "straight" downstrokes (on account probably of wash-ing back into the blood stream of dye which had diffused into the capillary walls) the vital red curves were always straight.   This was true whether the dye was injected in front of or behind the bulb, so as to have or not to have mixing comparable to that which occurs in the right heart in animal experiments.

The effect of different relationships between flow and volume.   Figure 4 shows three types of curves.   In the steepest, the flow is rapid in relation to volume, as in normal man and animals; in the intermediate curve, the flow is slower in relation to volume as in dogs under the influence of mor-phine; and in the flattest, the flow is very slow in relation to volume, as in cases of cardiac decompensation.   In all three cases, the downstroke is a straight line, and the flow is calculated accurately.   Since the curves from living animals correspond, until recirculation begins, with these curves, and since these curves are of the type which can be used in calculating the output and volume, they confirm us in our belief that the method is usable under a wide variety of conditions.

Estimation of volume in such a way as to check, in these experiments, the calculation based upon mean circulation time and flow, has proved very unsatisfactory.   The presence of stagnating blood in the lungs (8, 9, 10) makes any modification of the Welcker method inapplicable, since the latter measures total blood volumes, while we are interested here in actively circulating blood volumes.   Figures derived from comparing the dilution of the dye before and after it had mixed with the blood in the dog (see above) were variable and could at best be said to be not inconsistent with the figures derived from the mean circulation time.

CONCLUSIONS AS TO THE NATURE OF BLOOD FLOW THROUGH THE LUNGS.

From what has already been said, it can be gathered that multiplying the mean circulation time by the flow gives only the volume of actively circulating blood in the lungs. What blood remains stagnant in the lungs (an emergency reserve perhaps) (10) is not mixed with the dye and is hence not in the calculation.

One would expect that, since there is stagnant blood in the lungs, there would also be blood moving very slowly. From the above analysis, however, this blood is accounted for by simple prolongation of the time out to infinity or, for practical purposes, to some arbitrarily chosen figure. In other words, when the lungs are perfused, there is no evidence of two or more systems in which the volume flow relationships are different. In all
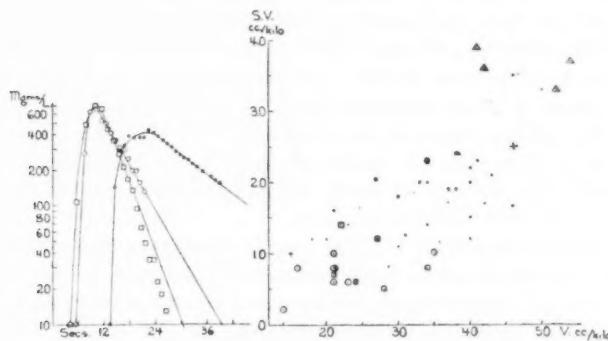
Fig. 4   Fig. 5

Fig. 4. Concentration curves of Brilliant Vital Red in blood perfused through the lungs of dogs.

Fig. 5. Relationships between stroke volume and intrathoracic blood volume in normal dogs, •; in morphinized dogs, △; dogs in hemorrhagic shock, ○; and dogs under the influence of barbiturates, □.

parts of the lung, the amount of blood led into it through the artery is nicely adjusted to the volume of the active vascular bed of that part. If the volume of the vascular bed in the left lung is three-fourths of that in the right lung, the flow to the left lung must be three-fourths of that to the right; otherwise the concentration curve would not be a "straight line." These nice adjustments of flow to volume do not exist in the peripheral vascular bed. One might expect this because of the varying vasomotor reactions in different parts of the peripheral system. Vasoconstriction will cut down the flow to a certain muscle or skin area without encroaching equally upon its vascular volume. When one takes consecutive samples from a superficial arm vein instead of following the usual procedure of arterial puncture, one finds that the concentration does not give a curve

resembling those discussed so far, but one that fluctuates up and down in quite an irregular manner.

ANALYSIS OF "V" FROM THE PHYSIOLOGICAL AND PATHOLOGICAL STANDPOINT. In the animal body, the factor V as calculated by multiplying the mean circulation time by the flow has a meaning which must be carefully defined. The experiments are usually performed so that the dye enters the portals of the heart immediately. In the dog the injections are made into the jugular. Experiments show (1, 3) that the dye is in the right ventricle in normal animals in one or two seconds. Similarly in normal man, injections are made into the antecubital vein of the upraised arm. The dye is seen to descend to the axilla as a "bolus" almost instantaneously and must enter the heart in normal man very soon thereafter. In the normal subject, then, the quantity V has an "anterior" of venous boundary at the point where the injected dye ceases to fall down the collapsed vein and mixes with the main stream. Anatomically this would be at the head of the column of blood raised by the venous pressure somewhere near the base of the axillary vein or in the subclavian. On the venous side V would include more than this. It would also include the blood in all the other great veins that reaches the heart in less time than it takes the median portion of the dyed blood to get there.

The venous boundary of V in the case of decompensation with markedly increased venous pressure would extend out the arm to the top of the column of blood supported by the increased venous pressure and to analogous points in the other branches of the venous tree. Blumgart and Weiss (11) have shown that the circulation time from the arm vein (arm in horizontal position) to the heart is increased in cases of decompensation. This would probably be true, but to a much less extent, when the arm is upraised and if so, would make V extend further out the venous tree in these cases than in normal cases.

On the arterial side V, would include all the blood out to the point of puncture and all the blood that gets out the other branches of the arterial tree in less time than it takes the (median) dyed blood to reach the sampling point. Between the arterial and venous boundaries of V are, of course, the right and left auricles and ventricles, and all of the actively circulating blood in the lungs.

The physical boundaries of V as we have defined them, are necessarily vague. Moreover, conditions which would cause the relative speeding or slowing of flow of one or more arteries or veins make these boundaries variable.

Our conception at the present time is that change in the blood capacity of the lungs is by far the most important cause for change in V. Thus certain decompensated cases which we have studied show a V increased from a normal of two liters to a value of four liters. Since there is probably

a relatively small increase in the vein (upright arm) to heart time, practically none in the left heart to artery time, and a great increase in the pulmonary circulation time (11), one is justified in assuming that the increased V is primarily due to pulmonary congestion.

In order to understand the situation, let us analyze the sequence of events involved in an increase of V. In dogs V usually increases when the heart rate is slowed, either spontaneously or under morphine. Since these conditions do not necessarily reduce the output, there is usually an increase in stroke volume. The essential correlation is, we think, one
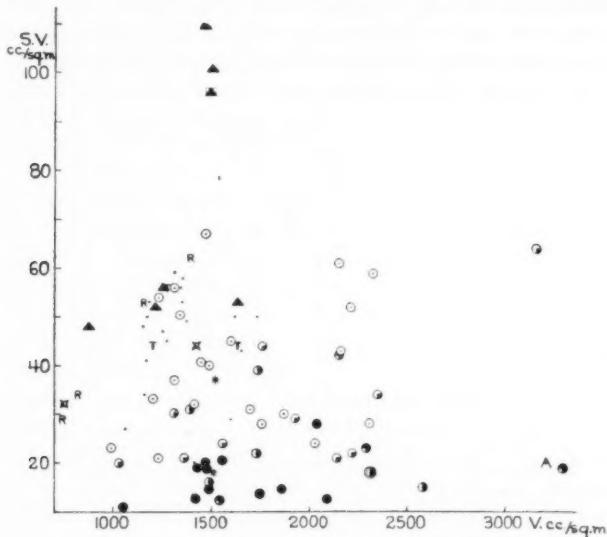
Fig. 6. Relationships between stroke volume and intrathoracic blood volume in man: normal .; cardiac decompensation ◔ ●; (the amount of blocking indicates the degree of decompensation); compensated cardiac cases ○; hyperthroidism, T; Raynaud's disease, R; pernicious anemia ▲.

between large stroke volume and increased V in normal individuals, and large diastolic size and increased V in the decompensated as well as the normal. This correlation is due to the increased pressure which it takes to fill the heart to the increased diastolic size. This increased pressure causes a congestion of the great systemic veins and an increase in the venous boundaries of V. The left heart also increases its diastolic size and there is here also a necessary increase in the filling pressure. To be effective, it must be transmitted from the right heart through the lung capillaries to the left heart. The studies of Drinker and his collaborators (12, 13) have substantiated the belief that the lung capillaries are subject

to remarkable increases in capacity by passive distention. On this basis, then, the increase in pressure which is necessary to increase the diastolic size of the heart (to increase the stroke volume in normal animals) is necessarily accompanied by an increase in the capacity of the lungs for blood and a decrease in their capacity for air (10, 12).

Increases in heart rate, on the other hand, which result from emotional excitement and are not accompanied by an increased output per minute, or even more strikingly, increases in heart rate from hemorrhage which are accompanied by a marked decrease in output, are both associated with a diminished stroke volume. Looked at from the above point of view, this reduced diastolic size (reduced stroke volume in normal animals) results from a decreased filling pressure on both sides of the heart. The consequences on the right side would involve a movement of the venous bound-
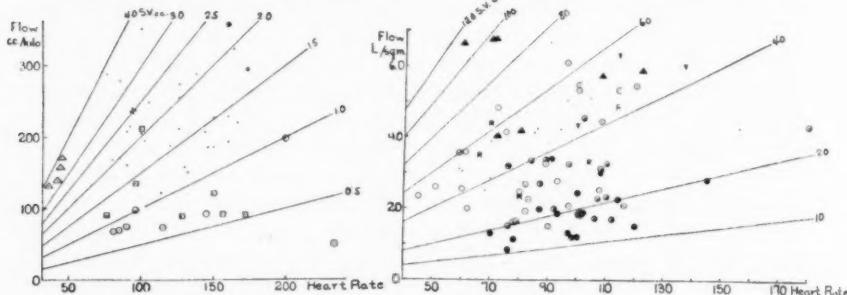
Fig. 7   Fig. 8

Fig. 7. Relationship between stroke volume, heart rate and cardiac output in dogs. Symbols as in figure 5.

Fig. 8. Relationship between stroke volume, heart rate and cardiac output in man. Symbols as in figure 6.

aries of V toward the heart, since the veins would be less engorged and the top of the column of blood supported by the venous pressure would not be so high. The difference on the left side would inevitably be greater. The elastic lung capillaries would tend to empty their contents against the decreased filling pressure of the heart and V would be reduced by this factor also.

Illustrative data. In confirmation of this way of looking at the situation we have the results of certain dog experiments, plotted in figure 5 so as to show the relation between stroke volume per kilogram and V per kilogram. In the morphinized dogs △ the large stroke volume is correlated with a large volume of blood in the heart and lungs (V). Several dogs after hemorrhage ○ and dogs which have been drugged with amytal and barbital □ show both stroke volume and the quantity V decidedly diminished. The normal undrugged dogs • are intermediate in position.

It is to be emphasized that, except for the natural scatter of the observations, the points in figure 5 group around a single correlation line. Factors which increase the stroke volume increase the intrathoracic blood volume.

When these same quantities are plotted (on a surface area basis) for man in normal and pathological conditions, figure 6, two groups are manifest. The first group includes those with normal cardiac function (• normal, ▲ pernicious anemia, R Raynaud's disease (14), T hyperthyroidism). In the second group ◐ • are the decompensated cases of cardiac disease. The amount of blocking indicates the degree of decompensation. An intermediate position is occupied by the cases ○ which have more or less regained their compensation. The decompensated group is characterized by a small stroke volume in relation to the amount of intrathoracic blood. The filling pressure of the right heart (venous pressure) is increased in decompensation. An increase in V is an index to the increase in the filling pressure of the left heart which would be expected to, and apparently does, accompany congestive circulatory failure.

In regaining compensation, the stroke volume may increase or remain fairly constant. The intrathoracic blood volume may decrease or not. The essential change seems to consist in assuming a more nearly normal relation between stroke volume and intrathoracic blood volume. There must be a reduction of diastolic size and hence of the filling pressure necessary for an adequate stroke volume.

In figures 7 and 8, the symbols are the same as in the preceding figures. The sectors indicate categories of stroke volume. The ordinates are cardiac output per minute and the abscissae are heart rate. In the dogs, heart rate has no evident relation to either stroke volume or output per minute. There is a tendency for the stroke volume of the dogs in hemorrhagic shock and under barbiturates to remain constant in spite of increases in heart rate. The same can be said of the normal human under our conditions. Nearly all of these subjects had a stroke volume per square meter between 40 and 60 cc. in spite of heart rates ranging from 60 to 120 beats per minute. This application of this principle—due to Henderson—must be restricted, as far as our data go, to subjects at rest and in the recumbent posture. What the findings will be under other conditions must await further information. In the cases of congestive failure it will again be noticed that there is little correlation between heart output and clinical condition. Cases with severe symptoms of the type which are usually referred to congestive circulatory failure may have larger outputs than those whose clinical condition shows marked improvement. How far this may be due to the entrance into the picture of non-cardiac symptoms which simulate those of failure and render the cardiac picture worse than it really is, and how far the actual mechanism of congestive failure is

in fact tied up with cardiac output per se, are problems of a most funda-
mental and difficult nature. They must await further experiment
and detailed clinical analysis.

SUMMARY

1. A mathematical equation is evolved (IV) which expresses the rela-
tionship between blood flow, the actively circulating intrathoracic blood
volume and the descending limb of the time-concentration curve of an
injected dye in successive arterial samples.

2. This equation contains a term, X, which cannot be directly deduced
from the above mentioned time-concentration relationship and describes
the degree of mixing between the dye and the actively flowing blood in
the thorax. Consequently, the flow through and the volume of the system
must be calculated from the time-concentration curve using separate
formulae which are given in the text (III, V, VI).

3. It is shown that the volume of actively circulating intrathoracic blood
cannot be calculated from the fastest circulation time. The time figure
which should be used is obtained by measuring the time between the middle
of the injection period to the center of gravity of the time-concentration
curve.

4. Experiments with glassware show that if the flows through two
parallel systems are each in the same proportion to their effective capacity,
samples of the mixed outflow give a time-concentration curve whose de-
scending limb conforms to equation IV. If the flow through one bulb is
greater in proportion to its effective capacity, the time-concentration
curve of the mixed outflow becomes a complicated summative curve whose
nature cannot be analyzed under the limitations of physiological
experimentation.

5. This situation is analyzed in detail and it is shown that if bulbs are
put in series before and after the bulbs in parallel (in the position of the
heart in relation to the lungs), the calculations of volume and flow are
facilitated.

6. When the lungs and one ventricle are perfused with blood, the injected
dye is washed out quite in accordance with equation IV; therefore, in each
of the parallel systems in the lungs (lungs, lobes, lobules) there must be the
same ratio between effective volume and flow.

7. It is shown that when vital red is used as the injected substance, no
appreciable dye diffuses through the walls of the lung capillaries and that
the measured perfusion rate checks very closely with that calculated from
the time-concentration relations of the dye in the perfused blood.

8. The quantity V is analyzed in detail as to its changing anatomical
boundaries, its physiological relationships to cardiac filling and pulmonary
congestion under various physiological and pathological conditions. Illus-

trative data are presented from 50 odd experiments on normal unanesthe-
tized dogs, morphinized dogs, and dogs in conditions of circulatory shock
as well as from 80 odd experiments on normal and ailing man.

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EFFECT OF OVARIAN SUBSTANCES ON EXCISED RAT UTERUS

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Frank (1) in 1925 advanced the idea that the ovarian function controls uterine motility, presenting, at the same time, evidence in support of this view. These results were in agreement with those of some of the earlier investigators. In 1923, Blair (2) demonstrated that the spontaneous contraction of the excised rat uterus, under favorable conditions, depends upon the period of the oestrous cycle in which the animal is killed. The rate of spontaneous contraction is rapid in dioestrus and slower in oestrus. Using strips of uteri from the domestic sow, Keye (3) in 1923 and Corner (4) confirmed these results. The exact relation of the rate of contraction of the uterus to the oestrous cycle has been questioned by Clark (5) and his co-workers. The present investigation was undertaken to determine whether these phenomena can be reproduced by the use of various ovarian preparations and substances on the excised uterus from which ovarian influence had been removed by ovariectomy.

Normal rats, whose vaginal smear history was noted by the method of Long and Evans (6), were ovariectomized. The complete removal of the ovary was determined by the continual presence of leucocytes in the vaginal smears. The uterus was excised under ether anesthesia and the horns separated at the cervix. Each horn was suspended separately in 50 cc. of oxygenated Ringer's solution in a bath automatically controlled and maintained at 37°C. throughout the experiment. Fifteen minutes elapsed after isolation of the uterus before movements were recorded by means of a standard Becker lever on a slow moving kymograph.

The extracts[1] were added in amounts varying from 0.1 to 1 cc. The effect of each preparation was recorded, the solution changed, the muscle washed with warm Ringer's solution and allowed to resume its normal rhythmic contractions. Where more than one preparation was used on

[1] The extracts used were Amnictin (Squibb) from amniotic fluid, Theelin (Parke-Davis) from urine of pregnant women, Whole Ovarian Extract (Lilly), Whole Ovarian Extract (Hynson, Wescott & Dunning), Ovarian Residue Extract (Hynson, Wescott & Dunning), Corpus Luteum Extract (Lilly) and Corpus Luteum Extract (Hynson, Wescott & Dunning) from dried corpus luteum.

the same tissue, the order was changed to prevent any possible sensitizing effect of the extract first used.

It was found that extracts made from ovarian tissue, whether whole ovary, residual ovary, or corpus luteum, decreased or inhibited the rate and rhythm of the spontaneous contractions of the isolated uterine muscle from ovariectomized rats. Amniotin from the amniotic fluid of cattle, and theelin, prepared from the urine of the pregnant female, apparently have no effect on the rhythmic contractions, in the amounts used.
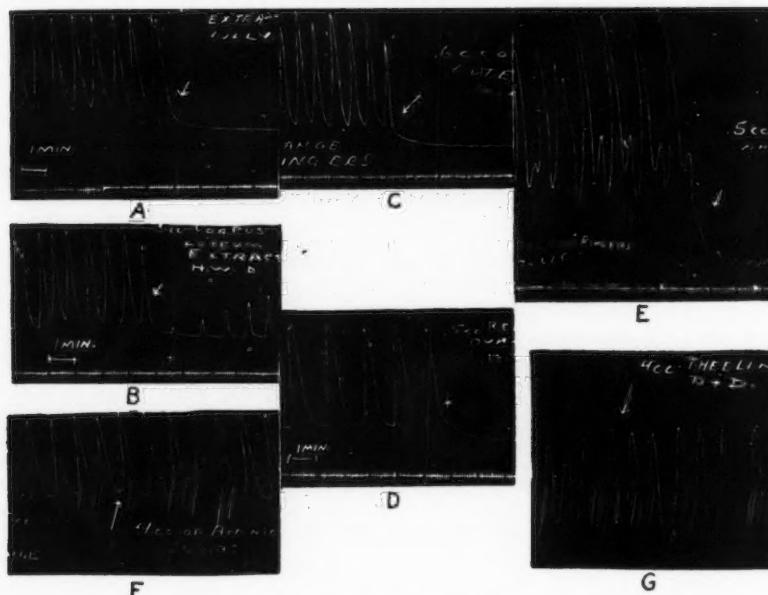
Fig. 1. Showing the influence of the various ovarian substances on uterine motility. A. Whole Ovarian Extract (Lilly), B. Corpus Luteum Extract (H. W. D.), C. Corpus Luteum Extract (Lilly), D. Ovarian Residue Extract (H. W. D.), E. Whole Ovarian Extract (H. W. D.), F. Amniotin (Squibb), G. Theelin (Parke-Davis).

Since the phenomenon of inhibition was demonstrable only with ovarian tissue extract, there suggests itself the possibility of the presence of a substance within the ovary, which affects the movements of the uterus and acts independently of the hormone which is responsible for the oestrous cycle. In view of the fact that corpus luteum extracts also exhibit this inhibition and since all the ovarian preparations used had corpora lutea present, we advance the following probability: that the corpus luteum elaborates this relaxing or inhibiting principle. This is in accordance with the fact dem-

onstrated by Wislocki in 1925, that uterine activity in the sow is very feeble during the presence of corpus luteum.

SUMMARY

The action of different sex hormone preparations upon the spontaneous rhythmic contractions of excised rat uteri was studied. It was found that all ovarian tissue preparations possess inhibitory action on the rate and rhythm of these contractions while those sex hormone preparations not prepared from the ovary were without action.

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THE EFFECT OF TEMPERATURE ON THE CONTENT OF SUGAR IN THE BLOOD OF THE ALBINO RAT

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Because of the conflicting reports of work that has been done on the relation between the environmental temperature and the concentration of blood sugar (Lee and Scott, 1914; Geiger, 1925, 1927; Horiuchi, 1924; M. O. Lee, 1926) it has seemed worth while to carry out a series of determinations upon the same animals kept for a period of time at ordinary temperature, 21° to 22°C., and then for similar periods at abnormally low and abnormally high temperatures respectively. Since it was impracticable to control the humidity of the room or cages in which the animals were confined no special attempt was made to do so, except to avoid extremes.

Albino rats were chosen for experimental animals because the rat is not prone to hyperglycemia and because previous experimental work has demonstrated that the concentration of blood sugar is fairly constant from day to day. Very few female animals were used in the course of the investigation since it was found in preliminary determinations that females show rather wide variations. This has likewise been reported by other workers in blood sugar and it seems probable that the period of oestrus influences blood sugar.

The food provided throughout the work was carefully standardized. "Old Trusty" dog cakes (meat 15 per cent, bone 10 per cent, wheat 75 per cent) were kept in the cage all the time and were the only food given for three days just preceding each analysis. Besides this, corn was fed once a week in such quantity as would be eaten up completely in approximately three days. Raw cabbage was likewise given once a week in such quantity as would be consumed in twenty-four hours.

The time covered by the experiments was seven weeks during which time the animals lived for two weeks under a low temperature, range −5 degrees to +7 degrees, and two weeks under a high temperature (in an incubator set at 34 degrees) and were sampled six times. Each time a sample was taken the animal was anesthetized with amytal.

On the whole the animals used remained in good condition throughout the work. In order to see just what effect the experimentation was having upon them, a group of eight were weighed at the conclusion of two series of determinations. The results are shown in table 1.

METHODS. A. *Analytic technique.* For this series of determinations Byrd's modification of the Folin-Wu method was chosen, primarily because of its simplicity, accuracy, and the small amount of blood required, the last factor obviating the danger of the results being vitiated by frequent sampling, as pointed out by Albritton (1924).

B. *Method of sampling.* After repeated trials of different methods and observing the effect of each upon the animal, it was decided to follow Kolmer's suggestion of snipping off the tip of the tail. Instead of filling the collecting pipette directly from pendent drops upon the tip of the tail it was found more satisfactory to allow the few drops to collect in the small concavity of a culture slide. From this the pipette could be filled with great precision. In this way the sample was easily secured and with no ill effects upon the animal. The wound always healed promptly and perfectly.

TABLE 1

Showing body weights of eight male rats anesthetized and sampled for blood six times during a period of seven weeks

NUMBER OF ANIMAL	WEIGHT AT BEGINNING OF PERIOD	WEIGHT AT END OF PERIOD	PER CENT OF LOSS OR GAIN
	grams	*grams*	
2	195	185	−5.1
5	215	212	−1.4
9	270	252	−6.6
27	265	270	+1.9
45	250	260	+4.0
50	145	138	−4.8
95	350	340	−2.9
140	215	210	−2.3

C. *Anesthesia.* No attempt was made to secure samples without anesthesia. Page (1923) demonstrated that isoamyl ethyl barbituric acid (amytal) would have no disturbing effect upon blood sugar. With the use of amytal the only precaution necessary is that of handling the animals at the time of injection so that excitement or emotional display is avoided. In only a few cases was the activity of the animal such as to arouse suspicion on this point, and in only a small number of these did the results appear to be affected.

EFFECT OF ENVIRONMENTAL TEMPERATURE UPON BLOOD SUGAR. Three series of blood sugar determinations were made. The first embodied only two different temperatures, that of the colony room, 22 degrees, and an outside winter temperature ranging from a little below the freezing point to approximately +7 degrees, (C), the average for the period being +2 degrees. The temperature at night occasionally fell to −5 and during

the daytime slightly higher temperature than the limit given was recorded, but this was exceptional during the month the animals were kept outside. Unfortunately the weather after the first week or two was such that respiratory trouble developed so that further work with this group was thought inadvisable.

TABLE 2

Comparison of the concentration of blood sugar of 20 animals living at ordinary room temperature and the same animals after living from 1 to 2 weeks "out of doors" in March weather

(In order to avoid the danger of vitiating the results through emotional hyperglycemia the body temperatures of the animals were not taken at the time of sampling, hence do not appear in the table.)

ANIMAL NUMBER	SEX	WEIGHT	CONCENTRATION OF SUGAR		CHANGE
			t = 22°C.	t = 0°C.	
		grams	*mgm. per 100 cc. blood*	*mgm. per 100 cc. blood*	
2623	M.	230	104	112	+8
2626	M.	240	111	115	+4
2631	M.	210	102	110	+8
2651	M.	200	119	124	+5
2653	M.	180	98	100	+2
4327	M.	140	140	110	+6
Means for males................			106.3	112	+5.7
2634	F.	150	110	119	+9
2635	F.	170	115	112	−3
2637	F.	150	109	112	+3
2638	F.	150	126	143	+17
2639	F.	150	73	113	+40
2640	F.	150	126	126	0
2642	F.	160	95	100	+5
4217	F.	200	89	110	+21
4243	F.	160	87	95	+8
4250	F.	140	90	102	+12
4254	F.	160	105	102	−3
4256	F.	160	100	101	+1
4262	F.	180	109	104	−5
4316	F.	180	107	122	+15
Means for females...............			103	111.4	+8.4

Two sets of determinations of blood sugar were made upon this group of animals, one before moving them to the outside, and another after two weeks' exposure to the outside temperature. The results appear in table 2.

Perhaps the most striking feature of these results is the much greater

range of variation among the females.   Moreover, it is only among the
females that there is any difference in the direction of the variation, three
of them showing a higher concentration at the higher temperature and
one no difference.   At the same time the averages of the sexes are not very
different, particularly with respect to the lower temperature.   These
results are the more significant since the body temperatures did not vary
materially throughout the work.

A second and third series of determinations embodied three different
temperatures, that of the colony room, averaging 21°C., a low temperature,
averaging near the freezing point (second series average +2, third series
average +1), and an incubator temperature, 34 degrees.   Outdoor winter

TABLE 3

*Showing the concentration average of blood sugar of 11 albino male rats under three
different environmental temperatures*

ANIMAL NUMBER	WEIGHT	MGM. SUGAR PER 100 ML. BLOOD			CHANGE	
		t = 1.5°	t = 20.5°	t = 34°	1.5–20.5	20.5–34
	grams					
2	195	113.3	104.6	90.5	+8.7	+14.1
5	215	114.3	103.0	95.9	+11.3	+7.1
9	270	117.2	108.0	97.6	+9.2	+10.4
27	265	117.2	104.0	100.5	+13.2	+3.5
43	350	114.9	104.9	99.6	+10.0	+5.3
45	250	118.6	105.4	93.9	+13.2	+11.5
50	145	122.4	100.9	92.8	+21.5	+8.1
95	350	116.3	109.9	98.7	+6.4	+11.2
140	215	118.8	110.0	102.0	+8.8	+8.0
303	160	112.1	108.4	98.6	+3.7	+9.8
504	140	119.0	96.4	98.9	+12.5	+2.4
Grand average..		116.7	104.2	97.2	+12.5	+7.0

exposure was used only in part this time, since electric refrigeration made
possible more constant conditions.   However, when outdoor exposure
was used in these series the animals were placed in a large rain proof case
which afforded adequate protection and made it possible to keep them out-
side indefinitely.   The lowest temperature recorded during low temperature
experiments was −8 degrees and the highest +9.   When the refrigerator
was used the temperature was held at +2 degrees with a thermostatic
range of 2 degrees, or the limits were +1 to +3 degrees.   While the
temperatures in outdoor natural refrigeration were more variable, the
average was almost identical with that secured with artificial refrigeration
and no difference could be detected in the effects upon the animals or in
the results obtained.

The incubator utilized was $4 \times 5 \times 8$ feet, electrically heated, and was set to hold a temperature of 34°C. which was about the highest that could be used without considerable discomfort to the rats.   At 34°, however, they can be kept in excellent condition for long periods of time.   Under these conditions they eat less, drink more water, and, as the results indicate, they mobilize much less sugar; while in the refrigerator just the opposite is true with respect to all three factors.

Table 3 records the average of the respective results of each individual which ran completely through both series.

THE BODY TEMPERATURE OF THE ALBINO RAT UNDER DIFFERENT ENVIRON-MENTAL TEMPERATURES.   It has been generally accepted that the concentration of sugar does not vary with the external temperature so long as the body temperature does not change.   In order to determine this point with

TABLE 4

Showing body temperatures of 11 male rats under different environmental temperatures

ANIMAL NUMBER	WEIGHT	REFRIGERATOR	COLONY ROOM	INCUBATOR
		t = +1°	t = 21° (av.)	t = 34°
	grams			
2	195	38.3	38.4	39.0
5	215	38.0	37.8	37.9
9	270	38.0	38.0	37.9
27	265	38.8	38.3	38.0
43	350	38.1	38.1	38.1
45	250	38.1	38.8	38.1
50	145	38.8	38.8	38.8
95	350	38.8	38.2	38.2
140	215	38.2	38.2	38.2
303	160	37.9	37.9	37.9
504	140	37.8	37.8	37.8

regard to the animals in question, their body temperatures were taken from time to time through the work under the different conditions described.   As already stated these temperatures were not taken on the days sugar determinations were made lest emotional hyperglycemia ensue. Instead, the body temperatures were taken a day previous to or subsequent to the blood sampling.

It has already been stated that during the first series of determinations the body temperatures of the animals did not vary perceptibly between the two conditions there included, namely, the colony room, 22 degrees, and outside winter, +2 degrees, the average of a large number of readings being 38 degrees.   Table 4 represents the average of two lots of animals in apparent good health at the time taken which, as stated, was near the time of their blood sugar determinations under the three sets of conditions given.

These results undoubtedly show that within the limits of temperature represented in the three sets of conditions the body temperature is unaffected by the environmental temperature. The body temperature does not fall when the environmental temperature drops to the freezing point. Under the low temperature much more food is consumed but less water, while under the high temperature much less food and a much larger quantity of water. This is altogether in harmony with the well established laws of physiology governing the regulation of body processes to meet changed conditions in the environment. While Lee (M. O.) reports that the body temperature of the albino rat was considerably lowered when the animals were exposed to environmental temperatures ranging near the Fahrenheit zero, no such reduction was found in the present series with temperatures ranging about the freezing point of water.

Likewise under the high temperature practically no change occurred; two animals showing a little higher average while two present a lower. It was not always possible to make all body temperature readings at exactly the same time of day so that a small amount of variation may be explained by the normal daily fluctuations. Another factor helpful to the animals in maintaining a normal temperature in the incubator was the fact that owing to its large size and the few animals in it at any one time together with the abundant ventilation, the humidity never rose noticeably above that of the adjoining room which varied around the 50 per cent mark during the time of experimentation.

SUMMARY AND CONCLUSIONS

1. The albino rat is an excellent experimental animal for blood sugar investigations since it is easily and cheaply kept, endures experimental conditions well, including frequent sampling, maintains a fairly constant level of sugar in the blood, and is less prone to emotional hyperglycemia than many others.

2. The micro-Folin-Wu method of blood sugar determination introduced by Byrd offers many advantages over most of the other methods now commonly in use for comparative studies. Its simplicity, accuracy, and rapidity, and the small amount of blood required are the chief advantages to recommend it.

3. Amytal is a satisfactory anesthetic for blood sugar work since it has in itself no disturbing effect upon the concentration of sugar and but very slight toxic effect. By keeping a layer of paraffin oil on the surface of solutions of amytal they may be kept indefinitely.

4. The body temperature of the albino rat does not vary materially as the environmental temperature changes, at least not within the limits of −8 to +34°C.

5. The concentration of sugar in the blood varies materially as the

environmental temperature changes. These two factors stand in an inverse relation to each other, as the temperature is lowered the blood sugar level rises; when the temperature is high the level of sugar is lowered. These changes obtain even within temperature limits which have no effect upon the body temperature.

These changes appear to be permanent, no readjustments occurring within three weeks, and they are probably in some way related to the metabolic readjustments necessary to meet the requirements of the animal under the changed conditions. The consumption of food is much greater when the animals are subjected to the cold environment, while the water used is less; while on the other hand, under abnormally warm conditions the consumption of food is much less and that of water much greater. It appears that under the low temperature the increased heat requirement is met by the mobilization of a larger quantity of sugar and that this increased metabolic action in the various tissues is accompanied by a high sugar level. On the other hand, under the high temperature the heat requirements of the body are greatly reduced and less sugar is mobilized and a lower level is maintained.

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FURTHER OBSERVATIONS ON THE NORMAL VARIATIONS IN ERYTHROCYTE VALUES IN WOMEN

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A study recently made by Smith (1931) showed that there were no significant variations in total numbers of erythrocytes and hemoglobin values during short periods of time. Since only the above mentioned values were considered in that work, the present study was made in order to see whether total cell volume, mean corpuscular hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration showed significant diurnal variations. It was found that subjects with a normal total red-cell count had higher total hemoglobin estimations and total cell volumes than those with low counts, while the calculated corpuscular values remained constant for all subjects. There were no significant diurnal variations in total numbers of erythrocytes, total hemoglobin content, total cell volume, mean corpuscular hemoglobin, mean corpuscular volume, or corpuscular hemoglobin concentration.

VOLUME DETERMINATIONS. *Technic.* Although most investigators[1] have preferred to use larger amounts of venous blood (4 to 10 cc.) for volume determinations, the Van Allen (1924-25) capillary hematocrit tube was chosen for the present study because it gave results that compared favorably with other hematocrit readings and was simpler to use when repeated estimations were made during the day. In the majority of the counts an International Clinical Centrifuge, radius of arm 7 cm., was used which was estimated to revolve at a maximum speed of 4000 revolutions per minute. The tubes were centrifuged until a constant volume was attained and the column of red-blood cells had reached a point of transparency. At this time also, the layer of white cells could be easily distinguished on top of the red ones. Centrifuging usually required about $\frac{3}{4}$ to 1 hour. Initial readings were made at the end of $\frac{1}{2}$ to $\frac{3}{4}$ of an hour and were continued at intervals of about 10 minutes until two consecutive ones showed a constant column for the red cells. In the first part of the work, 2 pipets were taken each hour, but later 3 or 4 pipets were averaged for the hourly values.

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[1] Extensive reviews of the bibliography are in Haden (1930) and Wintrobe (1930b).

The diluent at first used was 1.3 per cent potassium oxalate solution, but sodium oxalate was found to give results more nearly like those obtained with whole undiluted blood. In a series of 19 pipets containing sodium and 19 pipets containing potassium oxalate (1.3 per cent solution of each), the potassium dilutions averaged 6.4 per cent higher than the sodium oxalate readings. Solutions of various strengths of sodium oxalate (1.25 per cent, 1.3 per cent, 1.35 per cent, 1.4 per cent, 1.5 per cent, 1.6 per cent) were also compared with whole undiluted blood. A dilution of 1.3 per cent was chosen for all experiments reported in this study because values obtained by it were very close to those for whole blood, and because it has been used more frequently by other investigators.

The accuracy of the method was further tested by taking 12 successive samples of blood from 1 subject. The coefficient of variation for the 12 pipets was 2.8 per cent (table 1); for the first 5 pipets, 0.5 per cent.

TABLE 1

Volume readings

	PIPET 1	PIPET 2	PIPET 3	PIPET 4	PIPET 5	PIPET 6	PIPET 7	PIPET 8	PIPET 9	PIPET 10	PIPET 11	PIPET 12
Value (per cent)	36.3	36.3	36.6	36.1	36.5	37.9	37.0	35.0	38.0	37.8	36.5	39.0

Mean = 36.92 per cent
Standard deviation = 1.03 per cent
Coefficient of variation = 2.8 per cent

Results. The averages of all the day (12) and night (22) series of the low and the normal groups were calculated (table 2) and the difference between the means was significant according to its probable error, 4.00 ±0.81 per cent (n = 144, low group; n = 128, normal group). Like the total counts of erythrocytes and the hemoglobin values, the total volume per cent of red corpuscles remained relatively constant during the 8-hour periods (table 3). The difference between morning and afternoon was −0.67 ±1.07 per cent and between early and late evening +0.50 ±1.20 per cent.

MEAN CORPUSCULAR HEMOGLOBIN. The formula given by Wintrobe (1929) was used in calculating this value and is as follows:

$$\text{Mean corpuscular hemoglobin (micromicrograms)} = \frac{\text{Hemoglobin in grams per 1000 cc. blood}}{\text{Total cell count in millions per cubic millimeter}} = \gamma\gamma.$$

Results. A comparison of the means for the low and the normal groups (table 2) revealed that the difference between the means was less than 2

times its probable error, and was therefore not significant, $1.32 \pm 0.97\gamma\gamma$. A depression appeared in the day series in the afternoon which proved not

TABLE 2

*Comparative table, erythrocyte values in women**

	(1)	(2)	(3)	(4)	(5)	(6)	W¹	W²	O¹	O²
	AVERAGES, 14 SUBJECTS		NORMAL, 6 SUBJECTS		LOW, 8 SUBJECTS					
Number of determinations...............	272	144	128	72	144	72				
Total red-cell count, millions...........	4.47	4.52	4.69	4.75	4.28	4.30	4.93	4.78	4.75	4.30
Total hemoglobin, grams per 100 cc....	13.7	13.8	14.6	14.6	12.8	13.1	13.8	13.9	13.6	12.3
Total volume, per cent.	39.7	39.1	41.8	40.9	37.8	37.3	39.5	41.0	40.9	37.0
Mean corpuscular hemoglobin.........	30.6	30.5	31.1	30.7	29.9	30.4	28–29		28.6	28.6
Mean corpuscular volume.............	88.4	86.1	88.8	85.9	87.9	86.2	85.0		86.0	86.0
Mean corpuscular Hb concentration.....	34.5	35.2	34.9	35.6	33.8	35.1	35.0		33.3	33.3
Color index..........	1.07	1.07	1.09	1.07	1.04	1.06	0.98	1	1	1
Volume index........	1.03	1.01	1.04	1.00	1.03	1.01	0.93	1	1	1
Saturation index.....	1.04	1.06	1.05	1.07	1.02	1.05	1.05	1	1	1

* Averages for 14 subjects (6 normal, 8 low) from 12 8-hour day series and 22 8-hour night series. Of the 272 determinations (8 x 34), 88 were on subject F (low series) and 72, on subject K (normal series). Columns 1, 3, 5—all data were used. Columns 2, 4, 6—the data from series on 2 subjects, F and K, were averaged into 2 series each (1 day, 1 night) to equalize their value in relation to the other 12 subjects. W¹—data from Wintrobe, 1930a, 1931. W²—data from Wintrobe, 1930a. O¹, O²—data from Osgood and Haskins, 1931.

TABLE 3

Diurnal variations in erythrocyte values

	TOTAL ERYTHRO-CYTES	TOTAL HEMO-GLOBIN	TOTAL VOLUME	MEAN CORPUS-CULAR HEMO-GLOBIN	MEAN CORPUS-CULAR VOLUME	MEAN CORPUS-CULAR HEMO-GLOBIN CONCEN-TRATION
		grams	per cent	$\gamma\gamma$	c.μ	per cent
Difference between morning and afternoon	$-35,000$ $\pm 83,000$	-0.23 ± 0.46	-0.67 ± 1.07	-0.59 ± 0.81	-0.37 ± 1.88	-0.13 ± 0.89
Difference between early and late evening	$+37,000$ $\pm 95,000$	-0.03 ± 0.52	$+0.50$ ± 1.20	-0.19 ± 0.98	-1.63 ± 1.61	$+0.28$ ± 1.02

a real one since the difference between the morning and afternoon counts was about equal to its probable error, $-0.59 \pm 0.81\mu$ (table 3). Neither

was any change noticeable during the evening as the difference between the averages of early and late evening counts was much less than its probable error, $-0.19 \pm 0.98\gamma\gamma$.

MEAN CORPUSCULAR VOLUME. These values were calculated by the formula of Wintrobe (1929).

$$\frac{\text{Mean corpuscular volume}}{\text{(cubic micra)}} = \frac{\text{Total volume of packed cells per 1000 cc. blood}}{\text{Total cell count in millions per cubic millimeter}}$$

Results. The averages of all counts for normal and low groups may be seen in table 2. The study of these means showed that the difference between them was less than its probable error, and was therefore not real, 0.90 ± 1.91 c.μ. The patterns of the curves for the values of mean corpuscular volumes during the day did not change; neither was there a real difference when the data for morning and afternoon were treated statistically, -0.37 ± 1.88 c.μ. (table 3). Although the difference between the early and late evening averages was not significant according to its probable error, $(-1.63 \pm 1.61$ c.μ.) the pattern for all the composite curves showed a slight depression toward 9 and 11 p.m.

MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION. Wintrobe's (1929) formula has been used in calculating these values.

$$\frac{\begin{array}{c}\text{Mean corpuscular hemoglobin} \\ \text{concentration}\end{array}}{\begin{array}{c}\text{Proportion of hemoglobin in} \\ \text{average cell (per cent)}\end{array}} = \frac{\text{Hb in grams per 100 cc. blood} \times 100}{\text{Volume in cc. per 100 cc. blood}}$$

Results. Averages for day and night series in the normal and low groups are recorded in table 2. The difference between the averages of the mean corpuscular hemoglobin concentration in the normal and low groups was less than two times its probable error and was therefore insignificant, 1.35 ± 0.89 per cent. The pattern of the curves is practically unchanged during the 8-hour periods for both day and night series, and the differences between the means for the early and late parts of the day and evening are not significant, -0.13 ± 0.89 per cent and $+0.28 \pm 1.02$ per cent (table 3).

COMMENT. *Total numbers of erythrocytes.* The average total number of red-blood corpuscles in this study (about 4,500,000) was much lower than that given for normal women by previous investigators. Wintrobe (1930a) has summarized 7 studies on total counts and found 4,780,000 per c.mm. to be the average for 186 normal women between the ages of 17 and 30 years from different parts of the world. In the present study, the mean for the normal group was 4.69 millions per c.mm. when all determinations on 6 subjects were averaged (table 2, column 3), or 4.75 millions per c.mm. when subject K was treated as a single individual (table 2, column 4). This identified the normal group as used here, with the normal average of

Wintrobe, while the mean for the low group fell below 4,400,000 per c.mm. The difference between these normal and low averages (4,693,000 - 4,275,000) of the total erythrocyte counts (418,000 ±32,000 cells per c.mm.) was about 13 times its probable error and the variation was one of real meaning.

Hemoglobin content. The hemoglobin values determined by a Bausch and Lomb hemoglobin attachment on a Dubosc colorimeter were corrected by comparison with the measurement of the hemoglobin content of blood by the Van Slyke apparatus (table 2). The total hemoglobin content for the normal group was 14.58 grams per 100 cc. of blood; for the low group 12.78 grams, and for all subjects 13.78 grams. These estimations were similar to the average (13.91 grams) given by Wintrobe (1930a) for 232 women between 17 and 30 years of age. When the difference in total hemoglobin content of the normal and low groups was calculated it proved to be 5 times its probable error (1.81 ±0.36 grams per 100 cc.), and was therefore significant but not as clearly so as the difference in total numbers. This was probably due to the less accurate technique.

Volume determinations. The volume per cent for 6 (normal group) individuals (table 1, column 3) was 41.8 per cent when all determinations were considered, and 40.9 per cent (table 2, column 4) when subject K was regarded as one person. These values compared favorably with the 41 per cent that Wintrobe (1930a) gave as the result of a compilation of data for 200 subjects from various parts of the world. The difference between the volume percentage of the normal and low groups (41.8 per cent − 37.8 per cent) was about 5 times its probable error (4.0 ±0.81 per cent) and most likely a significant difference. A decrease of approximately 420,000 cells per c.mm. has therefore produced a diminution of about 4 per cent in volume percentage per 100 cc. of blood.

There seemed to be an exception to this generalization in subject K where the average for the day series of red-cell counts, although lower than that for the night, was accompanied by a higher value for total cell volume (44 per cent for the day, 42 per cent for the night series). These high volume determinations influenced the averages recorded in table 2, columns 1 and 3, so that slightly higher volume percentages accompanied lower erythrocyte totals. Data were obtained from 4 new day series on subject K and the new average, 42 per cent, confirmed the one previously obtained for the night series. However, the new figures changed values in table 2 so little that they were not included in the final data.

Color index, volume index, and saturation index. In each case, these indexes are based on a ratio of the blood values in question to the normal standard for the same age and sex group. Each one has a normal average value of 1 with a range from 0.85 to 1.15. The standards given by Osgood and Haskins (1931) were employed in the present study and in each in-

stance the results were between 1.01 and 1.09. This evidence indicates, therefore, that for the total numbers of erythrocytes, the corrected hemoglobin estimations and the volume percentage determinations, as obtained by the Van Allen capillary tubes, were within their normal limits (according to the standards used).

Mean corpuscular hemoglobin. The average value for mean corpuscular hemoglobin in table 2 was $30.5\gamma\gamma$ when the corrected hemoglobin estimation was used. Although the mean corpuscular hemoglobin content of the normal group was higher than that of the low group, nevertheless the difference between the two was less than 2 times its probable error and therefore not significantly different. The same results were secured when the corpuscular hemoglobin values were computed for figures given by Osgood and Haskins (1931).

Mean corpuscular volume. The mean corpuscular volume was stated by Wintrobe (1931) as about 85 c.μ with no significant differences between the sexes but with slightly higher values in those residing in the northern United States.   In the present study, all of the figures obtained were somewhat above that value (table 2).

The difference between the means of the cell volumes for the normal and low groups was proved insignificant because the difference was less than its probable error (0.9 $\pm$1.91 c.μ).

Mean corpuscular hemoglobin concentration. When the corrected hemoglobin estimations were used in the determination of corpuscular concentration, all of the figures obtained were about 35 per cent (table 2).   This was the value given by Wintrobe (1931) as the normal one.   The proportion of hemoglobin in the cell remained relatively constant throughout the study (tables 2, 3). Csáki (1921-22), Haden (1925), and Wintrobe (1930b) also emphasized the fact that the actual concentration of hemoglobin in the average cell remains very stable.

DIURNAL VARIATIONS. *Variations in total numbers of erythrocytes and hemoglobin content.* Since Smith (1931) discussed the work of previous investigators on the normal variations of erythrocytes and hemoglobin values, the main points only are summarized here.   One group of workers claimed that there were large diurnal variations and another that there were small variations within the limit of error for the method.   Smith (1931) found that the variations in hemoglobin and total numbers of erythrocytes remained fairly constant during the day and were not affected by rest, food or moderate activity.   It was noted, however, that the curves for the data of the 8 a.m. through 3 p.m. series showed a slight depression toward afternoon which was mathematically insignificant.   A similar slight depression occurred in the day series of this study but it also proved to be insignificant (difference = 35,000 $\pm$83,000 cells per c.mm). Even when the data from the present study were used with those of the previous paper (Smith, 1931) the depression was too small to prove real.

Variations in volume determinations.   Practically all of the work, except that done in connection with pernicious anemia and other blood diseases has tended to establish normal values by a study of many different individuals, and little has been said in the literature about normal variations in successive determinations on one person.   Haden (1923) and Ponder and Saslow (1930b) observed no diurnal variation in cell volume.   Keith, Rowntree and Geraghty (1915) reported that repeated volume determinations by the dye method on the same person gave practically identical results; by the hematocrit method, close but not the same estimations. Wiechmann and Schurmeyer (1926) considered that diurnal variations were present and Mills (1925) and Price-Jones (1920), (1930) found evidence for fluctuations of considerable size.   In 1920 Price-Jones calculated a difference of 0.6μ in cell diameter during the day, and in 1930 one of 0.2μ to 0.3μ.

The pattern for the day series showed a depression in the afternoon but this was so slight that it could not be proved significant, the difference between the morning and afternoon averages being less than its probable error (0.67 ± 1.07 per cent).   The conclusion may therefore be drawn that the volume percentage as determined by the Van Allen hematocrit method remains constant during the 8-hour periods studied.

Variations in mean corpuscular hemoglobin.   The estimated amount of hemoglobin in the cell is maintained at a fairly constant level during the 16-hour periods studied (difference between morning and afternoon is $0.59 \pm 0.81\gamma\gamma$ and between late afternoon and evening, $0.19 \pm 0.98\gamma\gamma$).

Variations in mean corpuscular volume.   Generally consistent values have appeared throughout the study in the estimated mean corpuscular volumes during the 8-hour series.   The results for these data may be contrasted with the observations on cell diameter made by Price-Jones (1920), (1930), who found a gradual increase in the diameter of the red-blood cells during the day.   The data in this study indicate no significant changes in mean corpuscular volume during the day or evening, even though the pattern of the curve showed an afternoon depression (difference between morning and afternoon counts, -0.37 ± 1.88 c.μ and between early and late evening, -1.63 ± 1.61 c.μ).   The absence of any record of change in cell volume during the day might be said to be due to the hematocrit method employed. Ponder and Saslow (1930a) stated that the hematocrit technic was unsatisfactory for accurate determination of cell volume.   Haden (1931), however, considered it the only practical way to measure cell size.   The variation in diameter (0.2μ 0.3μ or 0.6μ noted by Price-Jones) in a day should appear in even inaccurate methods of volume estimations if, as reported by Haden (1931), a change of 1μ in diameter in the red cell caused a modification of 44 per cent in cell volume.

Variations in mean corpuscular hemoglobin concentration.   The differ-

ences between the means for hemoglobin concentration during the sixteen hour periods studied also appeared insignificant according to their probable errors (difference between morning and afternoon counts, -0.13 ± 0.89 per cent, and early and late evening, $+0.28 \pm 1.02$ per cent).

SUMMARY

1. Two groups of subjects were studied, the first having a normal and the second, a lower number of erythrocytes.

2. The total number of cells, total hemoglobin content and total volume per cent were significantly higher in the normal than in the low groups, but the estimated mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration remained constant.

3. There were no significant diurnal variations in total number of cells, total hemoglobin content, total cell volume, estimated mean corpuscular hemoglobin, mean corpuscular volume, or mean corpusclar hemoglobin concentration.

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EFFECTS OF LOW ALVEOLAR OXYGEN AND HIGH ALVEOLAR CARBON DIOXIDE ON THE RATE OF FLOW OF CEREBROBROSPINAL FLUID[1]

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Recently Hertzman and Gesell (1928) studied the effects of low alveolar oxygen and other procedures on the hydrogen ion concentration of the cerebrospinal fluid of the dog. This paper deals with the effects of administration of gaseous mixtures low in oxygen or high in carbon dioxide on the rate of flow of cerebrospinal fluid of the dog. The animals were anesthetized with morphine and urethane and the flow recorded by the drop method from a cannula placed either in the spinal subarachnoid space or in the cisterna magna. The latter method was used in all except a few of the earlier experiments. The experimental gases were administered by means of rebreathing tanks of about 100 liters capacity, on each of which was mounted a Krogh spirometer. In the series of experiments in which constant artificial ventilation was employed, a battery of three of these tanks was used, one for high carbon dioxide mixtures, one for low oxygen mixtures, and one for room air. In the normal ventilation experiments only two tanks were used, the animal breathing room air directly from the room. However, in these experiments a gas meter provided with electrical contacts was interposed between the animal and the source of air by means of which a record of the volume of gas inspired was obtained. About fifty experiments were performed in all. Results of selected experiments are shown in figures 1 and 2. In figure 1 gaseous mixtures were administered with the chest intact. In figure 2 pneumothorax was established and physiological control of ventilation substituted by mechanical ventilation of constant rate and amplitude.

In figure 1 the uppermost record shows drops of cerebrospinal fluid. Below this is a spirometer record of pulmonary ventilation during the experimental procedure. Below this is a record of blood pressure in the femoral artery. Immediately below the blood pressure record is a record of volume of air inspired obtained by recording semi-revolutions of a gas meter of 4600 cc. capacity placed between the animal and the source of air.

[1] Preliminary report: Proc. Soc. Exp. Biol. and Med., 1929, xxvi, 831.
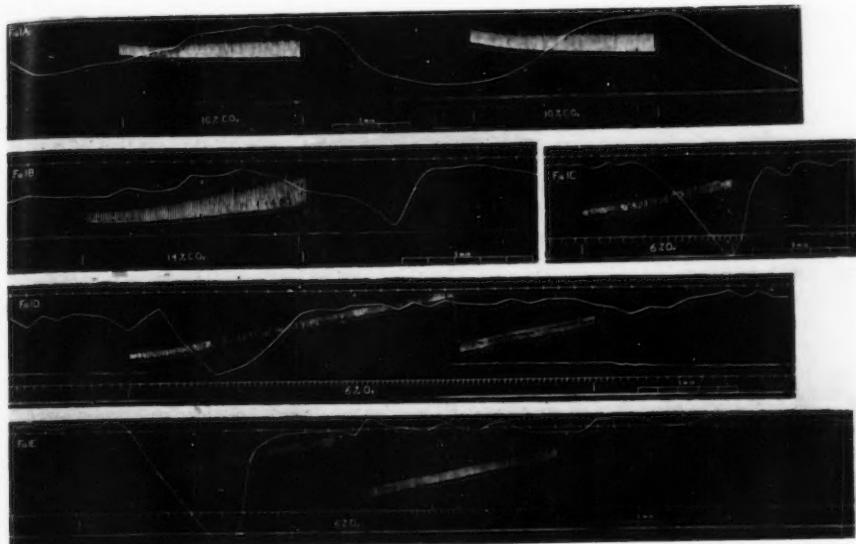
Fig. 1
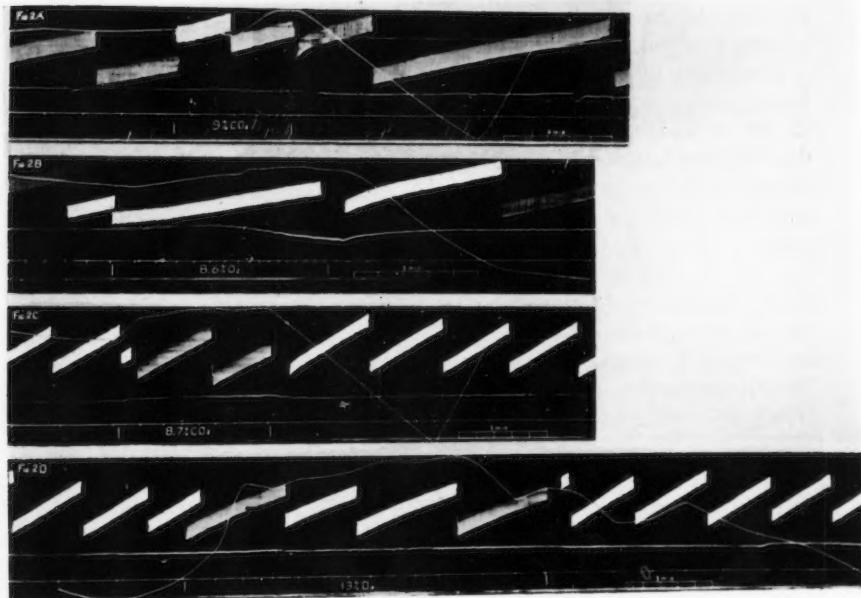
Fig. 2

Superimposed upon the graphic record is plotted the rate of flow of cere-
brospinal fluid on the ordinates against time on the abscissae. The in-
variable effect of the administration of gaseous mixtures high in carbon
dioxide (10–14 per cent CO_2, 21–26 per cent O_2) was a marked increase
in the rate of flow of cerebrospinal fluid. This increase appeared imme-
diately upon the administration of the gas, was very rapid at first and then
more gradual until the administration ended. In a few cases, as illustrated
by figure 1B, the rate of flow began to decrease slightly while the high
carbon dioxide mixture was still being administered, but in any case the
rate was still far above the normal level when the administration ter-
minated. This was true even though the administration continued as long
as twenty-five minutes. Upon readministration of room air the rate of
flow rapidly fell to the preadministrational rate. In some cases, as in
figure 1B, it first fell considerably below the normal level, later returning
to the preadministrational rate. In one experiment, at the beginning of
the administration of 10 per cent carbon dioxide the cerebrospinal fluid
was flowing at the rate of one drop in one hundred and eighty-five seconds.
After the administration had been continued for fifteen minutes the time
between drops had fallen to a minimum of sixty-two seconds. The ad-
ministration was continued ten more minutes, when the time between
drops had increased to ninety-five seconds. Thirteen minutes after re-
administration of room air the time between drops had increased to four
hundred and twenty-three seconds and nine minutes later had decreased
to two hundred and eighty-five seconds.

The effects of low oxygen (6 per cent) are somewhat more complex.
Low oxygen almost invariably caused a rapid and marked decrease in flow.
If the administration was sufficiently prolonged (14–41 minutes) the
decrease was followed by a rapid increase approaching and in a few cases
exceeding the preadministrational rate. If the administration of the gas
was still further prolonged the rate of flow changed relatively little, in some
cases showing a gradual and slight increase, in others a similar decrease.
In some of the experiments the initial decrease in flow was preceded by a
slight and very brief increase. The effects of readministration of room air
were quite variable. In most cases this readministration was followed
by a sharp decrease and a subsequent increase in flow to approximately
the previous rate, thus reproducing, though usually in lesser degree, the
effects produced by administration of the low oxygen mixture. However,
almost as often the order was reversed, the increase preceding the de-
crease, while in a few cases the return to room air seemed to have no very
marked effect on the flow of cerebrospinal fluid. If the administration of
low oxygen was continued for a shorter time (5–8 minutes) as in figure 1C,
so that room air was readministered during the initial decrease in flow, the
readministration of room air was followed immediately by a rapid increase
to or above the preadministrational rate.

In the artificial ventilation experiments, some of which are shown in figure 2, both room air and the experimental gases were administered from rebreathing tanks. The gas meter was omitted, the record immediately below the blood pressure tracing showing drops of cerebrospinal fluid. When gaseous mixtures high in carbon dioxide (8–12 per cent CO_2, 21–28 per cent O_2) were administered with constant artificial ventilation the effect on the flow of cerebrospinal fluid was similar to that obtained with the ventilation under physiological control, viz., a marked and well maintained increase in rate. Upon readministration of room air the rate of flow usually rapidly fell considerably below the preadministration level and then more gradually returned toward, though in most cases did not reach the normal.

However, the effects of administration of low oxygen mixtures (6–13 per cent) by constant artificial ventilation differed markedly from those obtained under normal ventilation. When such mixtures were administered by artificial ventilation there invariably occurred a prompt and marked increase in the rate of flow of cerebrospinal fluid. As in the case of the carbon dioxide mixtures, if the administration was sufficiently prolonged the rate of flow might begin to decrease slightly before the end of the administration, but in practically every case it was still considerably above the normal when the administration ended, even though, as in one experiment, the administration was continued as long as forty minutes. Upon the readministration of room air the flow quickly returned to the normal rate, in some cases falling considerably below it and then returning toward it. The results of these experiments in which artificial ventilation was employed are in essential agreement with those previously reported by Dixon and Halliburton (1913, 1914).

That the method of recording the rate of outflow of cerebrospinal fluid from a cistern cannula may yield unreliable data has been pointed out by various workers (Weed and Cushing, 1915; Becht, 1920). However since, as Weed (1922) has shown, the manometric method also fails in many respects, it was believed that, with the use of extreme caution in the analysis of results, the outflow method would be the more satisfactory for these studies. The chief objection to the outflow method is that an increase in intracranial pressure due to vascular changes or respiratory movements will force out preformed fluid leading to the erroneous conclusion that there has been an increased formation of fluid. That the flow of fluid is readily influenced by respiratory movements and by blood pressure there is no doubt. With the chest intact the drop of fluid on the end of the outflow tube is seen to rise markedly with each inspiration and fall with each expiration while superimposed upon these movements are smaller oscillations of the drop synchronous with each heart beat. It might be suggested then that the various increases in flow observed were due to the in-

creased respiratory movements. However, in many cases low oxygen caused as marked an augmentation of respiration as did high carbon dioxide, while the former, with ventilation under normal control caused a decrease in flow. In the artificial ventilation experiments there was no oscillation of the drop with respiration, and it is difficult to see how respiratory movements, which usually occurred during the administration of the experimental gas though absent during room air, could appreciably affect the intracranial pressure with the chest widely open. Nevertheless, in order to eliminate completely such a possibility, in several artificial ventilation experiments, after the effects of high carbon dioxide and low oxygen mixtures had been determined, the animals were completely curarized preventing any respiratory movements, and the observations repeated. In every case the increased flow produced both by carbon dioxide and low oxygen appeared just as marked as before. Figures 2C and 2D show observations upon a curarized animal.

Let us now consider the possibility that the changes in flow which we have observed were due solely to vascular changes. It is the generally accepted view that vasomotor fibers to the vessels of the brain, if present, are relatively unimportant, vascular changes being largely passive, dilatation resulting from increased general blood pressure and vice versa. We would expect then that in general a rise in blood pressure would result in an increased flow of cerebrospinal fluid simply due to the forcing out of preformed fluid. This has been found to be the case. That the effect of adrenaline in increasing the flow or pressure of cerebrospinal fluid is due to such an action has been shown by Becht (1920) and others. The increase in the flow during the administration of high carbon dioxide mixtures was usually accompanied by a rise in arterial blood pressure but this was not invariably the case. There were several experiments in which, as in figure 1A, there was no rise in blood pressue, and even one or two in which there was a progressive fall throughout the administration. When low oxygen was administered, whether by normal or artificial ventilation there was almost invariably a marked rise in arterial blood pressure, this rise coinciding in the normal ventilation experiments with a decided decrease in flow of cerebrospinal fluid. If room air was readministered while the flow was still diminishing there occurred a fall in blood pressure associated with a rise in the flow of cerebrospinal fluid. If the administration of low oxygen was prolonged sufficiently the rise in blood pressure was usually succeeded by a fall coinciding, in many cases, with an increase in the flow of cerebrospinal fluid. In the artificial ventilation experiments, as has been pointed out, low oxygen caused an increase in flow, and in some cases, as illustrated by figure 2B, this increase in flow became most marked after the blood pressure had fallen considerably below normal.

It seems unlikely that under these conditions the increased flow could be explained by vascular changes. Since there appears to be a complete lack

of correlation between rate of flow of cerebrospinal fluid and arterial blood pressure, the changes in flow which we have observed cannot be due to changes in intracranial pressure consequent upon changes in arterial pressure.   Becht (1920) has pointed out that intracranial venous pressure may vary independently of general arterial pressure and believes that it may markedly affect the pressure and flow of the cerebrospinal fluid.   Dixon and Halliburton (1914) believe that venous pressure has very little effect upon the pressure of cerebrospinal fluid but admitting Becht's contention, it is difficult to believe that the changes which we have observed could be explained on that basis.

We believe then that the changes in rate of flow of cerebrospinal fluid which we have recorded represent in large measure changes in rate of formation of the fluid rather than simply changes in rate of output due to mechanical factors operating within the cranial cavity, realizing that the latter mechanism may complicate the picture.   In this connection the marked similarity between the effects of high carbon dioxide and low oxygen administered with normal ventilation upon the rate of flow of cerebrospinal fluid and upon the rate of salivary secretion by the submaxillary gland as reported by Eddy (1929) seems worthy of mention. Eddy found that high carbon dioxide caused a marked increase and low oxygen a decrease, followed after prolonged administration by an increase in the rate of secretion by the submaxillary gland.   Gesell (1928) on the other hand found an increase in the rate of flow of lymph with both high carbon dioxide and low oxygen, together with increased turbidity indicating increased permeability of the vascular membranes involved.   In view of this our results might be taken to support the generally accepted view that the cerebrospinal fluid is a true secretion rather than a simple filtrate or dialysate.

The effects of high carbon dioxide mixtures whether administered with artificial or normal ventilation may be explained on the basis of increased acidity of the cells of the choroid plexus.   Gesell and his co-workers (Gesell and Hertzman, 1927; Gesell, Brassfield, Krueger, Nicholson and Pelecovich, in press) have found that low oxygen when administered with ventilation under physiological control produced a marked initial increase in alkalinity of the arterial blood which in some cases was followed by an increasing acidity while the gas was still being administered.   Their calculations indicate that this alkalinity of the arterial blood may be associated with increased alkalinity of the tissues.   Hertzman and Gesell (1928) have also shown that low oxygen with normal ventilation causes an increased alkalinity of the cerebrospinal fluid.   On the other hand, Gesell and Hertzman (1927) and Gesell, Krueger, Gorham and Bernthal (1930) have found that if low oxygen is administered with constant artificial ventilation this alkaline change in the arterial blood is less marked and is probably associated with increasing tissue acidity.   Assuming then that increasing acidity of the cells

responsible for the formation of the cerebrospinal fluid stimulates their activity and vice versa, we can explain the initial decrease in flow when low oxygen is administered with normal ventilation as due to the decreased acidity and the subsequent increase with prolonged administration as due to the gradually increasing acidity. It may be that the brief and slight increase in flow sometimes found to precede the initial decrease is associated with an increasing tissue acidity until the subsequent alkaline effects of increased ventilation predominate. Similarly when low oxygen is administered with artificial ventilation the increase in flow may be due to a progressive increase in tissue acidity.

SUMMARY

Effects of administration of gaseous mixtures high in carbon dioxide and low in oxygen upon the rate of flow of cerebrospinal flow of the dog were studied.

High carbon dioxide mixtures whether administered with the ventilation under physiological control or with constant artificial ventilation caused a decided increase in the rate of flow of cerebrospinal fluid.

With normal ventilation low oxygen caused a marked decrease in the flow of cerebrospinal fluid followed in prolonged administrations by an increase approaching the normal rate. With constant artificial ventilation low oxygen caused an increase in the flow of cerebrospinal fluid.

We believe that the changes in flow which we have observed are due primarily to changes in the rate of formation of the fluid, though realizing that they may be complicated by other factors.

The fact that the flow of cerebrospinal fluid in its reponse to the administration of these gases resembles salivary secretion but differs from lymph flow suggests a secretory mechanism of formation of the fluid.

We have suggested an explanation of these changes in flow on the basis of changes in acidity of the cells of the choroid plexus, assuming that increasing acidity increases the rate of formation of cerebrospinal fluid, and vice versa.

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THE NORMAL PERIOD OF SUBMERGENCE FOR THE HIPPOPOTAMUS

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During a visit to Hagenbeck's Zoölogical Garden near Hamburg, in the summer of 1930, I noticed two young hippopotamuses (H. amphibius Linn.) in a large pool of water resting quietly near the bottom but within easy sight. From time to time, they lifted their nostrils above the water and took breath. The opportunity seemed propitious for recording the breathing rates. I was accompanied by Dr. Benjamin Kropp and between us we took a continuous series of readings on the two animals, till a group of visitors arrived and disturbed them.

The two hippopotamuses were each about two meters long and were resting close to the edge of the pool. It was comparatively easy to measure with an ordinary watch, provided with a second-hand, the periods of submergence between respirations and thus to gain some idea of the respiratory rate in the resting animals. Of the seven records taken on the first hippopotamus, the shortest was 28 seconds, the longest 1 minute, 14 seconds, and the average 52.1 seconds. Of the same number taken on the second animal, the shortest was 23 seconds, the longest 1 minute, 22 seconds, and the average 49 seconds. The general average of these two series was 50.6 seconds. It may, therefore, be said that a small resting hippopotamus will rise to the surface for breathing on the average about once in 50 seconds.

This determination is somewhat longer than that attributed to Colin by Babák (1921, p. 958), namely, three to seven respirations in a minute, which implies respiratory intervals of from 20 to about 9 seconds. It agrees, however, very closely with Vever's statement (1926, p. 1099) based on observations in the London Zoölogical Garden, that hippopotamuses remain submerged on the average 40 seconds. This author noted that suckling young come up at intervals of 20 seconds for breath. He also gives as an extreme interval for the adult animals three minutes, a figure very near to those observed by Selous in the open field, as quoted by Amédée-Pichot (1919, p. 297), namely, 40 seconds to 4 minutes and 20 seconds, with two to two and a half minutes as the usual period. These records are in fair agreement with Brehm's general statement (1877, p. 574) of a maximum of four to five minutes.

Brehm calls in question all claims to longer periods. These include such records as that of a maximum of ten minutes, as quoted by Beddard (1902, p. 274) from Baker, and of a quarter of an hour, as stated by Bartlett (1872, p. 821). Under the same head would come the very long period of half an hour, as given by Pocock (1918b) on the basis of Keeper Topping's memory and of 29 minutes from the same author (1918a) as observed by Keeper Robinson. According to Robinson a hippopotamus in the London Zoölogical Garden, after having been scared, plunged under water and remained there in clear view without bringing its nostrils to the surface for 29 minutes as timed by a watch. This animal, as already stated, had been scared and would be expected, therefore, to remain submerged a long time, but notwithstanding this fact, as well as the circumstantial nature of the account, it is extremely difficult to credit this statement as unqualifiedly true. I am distinctly inclined to agree with Brehm that all such declarations call for rigorous confirmation.

To me it seems probable that the hippopotamus is an animal not especially adapted for extended life under water, as for instance the manatee. The average period of submergence of the manatee, when quiescent, is for small individuals about four and a half minutes and for large ones about twelve minutes (Parker, 1922). These periods characterize mammals highly adapted to aquatic life and are strikingly longer than the 50 seconds characteristic of the resting hippopotamus. This animal appears not to be specially adapted to life in the water but in this respect it is more closely related to such creatures as man, whose extreme limits under water are ordinarily three to four minutes. All such determinations of course presuppose the oxygen pressure in the atmosphere at approximate sea-level. Schneider's recent noteworthy observation (1930) that when a human being is freely provided in advance with pure oxygen the breath may be held fifteen minutes shows how enormous is the reserve capacity in this respect, even though in normal life such reserve can never be called upon.

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OBSERVATIONS ON THE EXTERNAL WORK OF THE ISOLATED TURTLE HEART[1]

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PART I. THE CALCULATION OF THE EXTERNAL WORK. The external work of the heart can be measured by determining the potential and kinetic energy imparted to the blood leaving the heart. It has been customary in the past to use mean pressure and mean velocity in calculating this work on the assumption that the error introduced by using these values is small and insignificant. This assumption has been made in Evans' formulae (1915, 1918). Similarly, Fahr (1927), while using an integration for the determination of the potential energy, employed mean velocity in calculating the kinetic energy. Frank's elaborate formula (1898) for the calculation of the total work of the heart, which avoids the use of mean pressure and velocity, has not been used except in part by Straub (1917) who constructed his work-diagrams from the first two terms of this equation.[2]

Frank (1898) stated that the error introduced by using mean values in the calculation was about 10 per cent. However, no one has investigated whether or not the error introduced in calculations of work by using mean pressure and mean velocity remains constant and small under most experimental conditions. This was attempted in the present research.

To simplify the problem the isolated perfused spontaneously beating turtle ventricle was employed. The arrangement has already been described and shown diagrammatically in figure 1 of a previous report (1930). With this arrangement the contraction of the turtle heart could occur under nearly isotonic conditions by connecting it to the large reservoir, E. The contraction could occur under auxotonic conditions to varying degrees by connecting the heart with tube G or H.[3] The initial volume of the heart

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[2] The various formulae, except Frank's elaborate one, are assembled in an article by Wiggers and Katz (1928).

[3] These tubes were 5 and 1 cc. pipettes respectively. Each cubic centimeter had a height of 3 cm. in the former and 13.5 cm. in the latter.

was also easily adjusted by means of reservoir E. Since the perfusion fluid contained only small quantities of oxygen, fatigue of the heart would eventually develop during the course of an experiment. The data for calculating the external work were obtained from pressure and volume curves recorded simultaneously without parallax on bromide paper.[4] Curves were obtained in six preparations, at different diastolic sizes under the three conditions of emptying before and after fatigue had developed.

The following formulae were used to calculate respectively the approximate external work of the heart (employing mean pressure and mean velocity) and the true value of the external work of the heart:

$$W = PV + \frac{Mv^2}{2g} \tag{1}$$

where W is the external work of the heart calculated as the pressure and kinetic energy possessed by the blood as it leaves the heart; P is the mean pressure above the zero level of the system near the opening of the aortic cannula during ejection; V, the volume ejected; M, the weight ejected; v, the mean velocity of the blood at the opening of the aortic cannula during ejection;[5] and g has the usual significance, viz., 980 cm./sec.[2]. And

$$W = \int_{T_0}^{T_1} P dV + \int_{T_0}^{T_1} \frac{v^2 dM}{2g} \tag{2}$$

where W and g have the same significance as in formula (1); T_0 and T_1 mark the beginning and end of ejection, respectively; P and v are the pressure and velocity, respectively, at the opening of the aortic cannula, of each increment of volume (dV) and weight (dM) ejected.[6]

[4] In the preliminary experiments an attempt was made to record the velocity of the blood directly by leaving the side tube of the oncometer open, but the difficulties encountered in obtaining such a curve accurately led to the abandonment of this method (cf. Frank, 1908).

[5] In reality v should be the difference between the mean velocity of blood ejected at the opening of the cannula and the mean velocity of the blood in the heart before ejection. Since the latter may be taken as equal to zero, the value of v becomes the mean velocity at the opening.

[6] To make the formula more accurate and comparable with Bernoulli's theorem cognizance should be taken also of the change in energy due to a shift in position. This would be determined as follows:

$$\int_{T_0}^{T_1} H dM$$

where H is the vertical height to which each increment of liquid is lifted; the other symbols have the same meaning as in formula (2). It is not practical to determine the energy of position by this formula. A fairly close approximation of this energy form can be obtained by HM where H is the height of the opening of the cannula

Formula (1) is comparable to the formulae using mean pressure and mean velocity. V, the volume ejected, in the first term on the right of the formula was determined in cubic centimeters as follows:

$$V = O K_V \tag{3}$$

where O is the vertical height of the volume curve change during ejection in millimeters; and K_V is the calibration factor converting millimeters of curve height into cubic centimeters of actual volume. The calibration factor K_V was determined after the heart ceased to beat by means of pipette L, which was graduated in cubic centimeters (c.f. fig. 1 of Katz, 1930).

The mean pressure, P, in formula (1) was obtained in centimeters of water as follows:

$$P = \frac{A_p K_p}{L} \tag{4}$$

where A_p is the area in square millimeters between the pressure curve and zero pressure during ejection; L, the length in millimeters of the ejection period; and K_p is the calibration factor converting millimeters of curve height into centimeters of water. A_p was determined with a planimeter on $2\frac{1}{2}$ fold enlarged tracings of the pressure curve. The calibration factor K_p was obtained by raising the filling reservoir a known height and dividing this height by the millimeter change recorded on the bromide paper.

The mass ejected, M, in formula (1) may be taken as equal to V when measured in grams since the specific gravity of the saline used for perfusion was considered as 1.00 which introduces no significant error. The specific gravity of the 0.7 per cent saline used is 1.005. M, therefore, actually equals 1.005 V. If blood had been used the correction would become important.

The mean velocity, v, in formula (1) was measured in centimeters per second as follows:

$$v = \frac{V}{xt} \tag{5}$$

where x is the cross section area of the opening of the aortic glass cannula and in these experiments equalled 0.0452 sq. cm.; t is the duration of ejection in seconds; and V has the same significance as in formula (3).

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above the center of gravity of the heart's cavity and M is the weight of fluid ejected. In these calculations the energy of position has not been included in order to make formula (2) comparable to formula (1). H in these turtle experiments was about the order of $\frac{1}{2}$ cm. so that this energy could be computed in gram-centimeters with little error by taking $\frac{1}{2}$ the volume ejected in cubic centimeters.

The values of P and V used here were chosen to make them comparable with formula (2) since they are the true arithmetical means of the values integrated by the latter formula. They are not equivalent to the values most commonly employed. For example, P is often taken as the mean of the maximum systolic and minimum diastolic pressures; on the other hand, P is sometimes chosen as the recorded mean pressure of the entire cycle, viz., as inscribed by a mercury manometer in the mammal. These values of P are not equivalent to the mean used in the present computations, but are distinctly lower.

In this research, the difficulties in determining the mean velocity introduced by a variable cross section area in the exit tube of the heart was avoided by the use of a glass cannula. Furthermore, most attempts to calculate the mean velocity ignore the fact that blood is ejected only over part of the cardiac cycle so that the values for mean velocity calculated on the basis of the entire cycle are too low and introduce a considerable error in the computations as Evans (1918) has shown. In determining mean velocity in the present computations, the duration of the ejection period was used instead of the entire cycle, cf. formula (5).

The calculation of the integrals in formula (2) was accomplished by using geometric constructions. The first term, giving the pressure energy, was obtained by plotting the pressure in millimeters as ordinate against the volume in millimeters as abscissa during the whole time of ejection. The area beneath the curve was measured in square millimeters with a planimeter and the pressure energy obtained in gram-centimeters as follows:[7]

$$E_p = \int_{T_0}^{T_1} P dV = A_{pV} K_p K_V \tag{6}$$

where E_p is the pressure energy form of the external work; A_{pV} is the area beneath the constructed pressure-volume curve; and the other symbols have the same meaning as in the previous formulae.

The second term, giving the kinetic energy, was obtained by plotting the square of the change in volume in millimeters per 5 mm. as ordinate against the volume in millimeters as abscissa for each increment of time during ejection.[8] A slide rule was used in calculating the square of the volume change. The area beneath this curve was measured in square

[7] An insignificant error is introduced by the fact that a tiny amount of the ejected fluid enters the pressure manometer as its rubber membrane bulges. In addition, there is another error in that the manometer cannula was not placed at the opening of the aortic cannula but a little distance in front of it.

[8] The change in volume was closely approximated but not actually measured by this method. The error introduced by the use of the volume ejected for the weight of fluid ejected is negligible since the specific gravity of the saline is 1.005.

millimeters with a planimeter and the kinetic energy obtained in gram-centimeters as follows:

$$E_k = \int_{T_0}^{T_1} \frac{v^2 dM}{2g} = \frac{A_{VM} \, K_V \, (K_E)^2}{2g} \tag{7}$$

where E_k is the kinetic energy form of the external work; A_{VM} is the area beneath the constructed volume flow-volume curve; K_E is the calibration factor converting volume flow in mm./5 mm. to velocity in centimeters per second; and the other symbols have the same significance as in the previous formulae.   The calibration factor, K_E, was obtained as follows:

$$K_E = \frac{K_V L}{5 \, xt} \tag{8}$$

where the symbols have the same significance as in previous formulae.

The method used to calculate the external work from the foregoing formulae and constructions might be made clearer by reporting the computations in a single case.   For example, curve 3a of preparation T28 shown in figure 2 of a previous report (1930), was enlarged $2\frac{1}{2}$ fold and retraced on millimeter coördinate paper by means of a baloptican as described by Rapport and Ray (1927).

Measurement showed that the height of the volume curve, O, which is the volume ejected, was 134 mm.   Calibration of the volume recorder had shown that 1 mm. on the enlarged tracing was equivalent to 0.015 cc. $= K_V$.   Hence the volume ejected according to formula (3) was 2.01 cc. $= V$.

Calibration of the pressure recorder showed that 1 mm. on the enlarged tracing equalled 0.27 cm. of $H_2O = K_p$.   The duration of ejection on the enlarged tracing was found to be 50 mm. $= L$.   The area on the enlarged curve between the pressure curve and zero atmospheric pressure during ejection was found by planimeter to be 2950 sq. mm. $= A_p$.   Hence, the mean pressure during ejection according to formula (4) was 15.93 cm. of $H_2O = P$.   Therefore the pressure energy form of the external work, PV, was 32.0 gm-cm.

Now $M \leftrightharpoons V = 2.01$ grams.[9]   Computation showed that 1 second on the enlarged tracing was equal to 55 mm.   Hence, the duration of ejection of this curve was 0.911 second $= t$; and the mean velocity during ejection, v, at the mouth of the cannula, whose cross section area equals 0.0452 sq. cm. was, according to formula (5), 48.9 cm./ sec.   Therefore, the kinetic energy form of the external work, $\dfrac{Mv^2}{2g}$, was 2.45 gm-cm.; and by formula (1) the external work, W, as determined by use of mean values was equal

[9] The error introduced by ignoring the specific gravity is $\frac{1}{2}$ per cent.
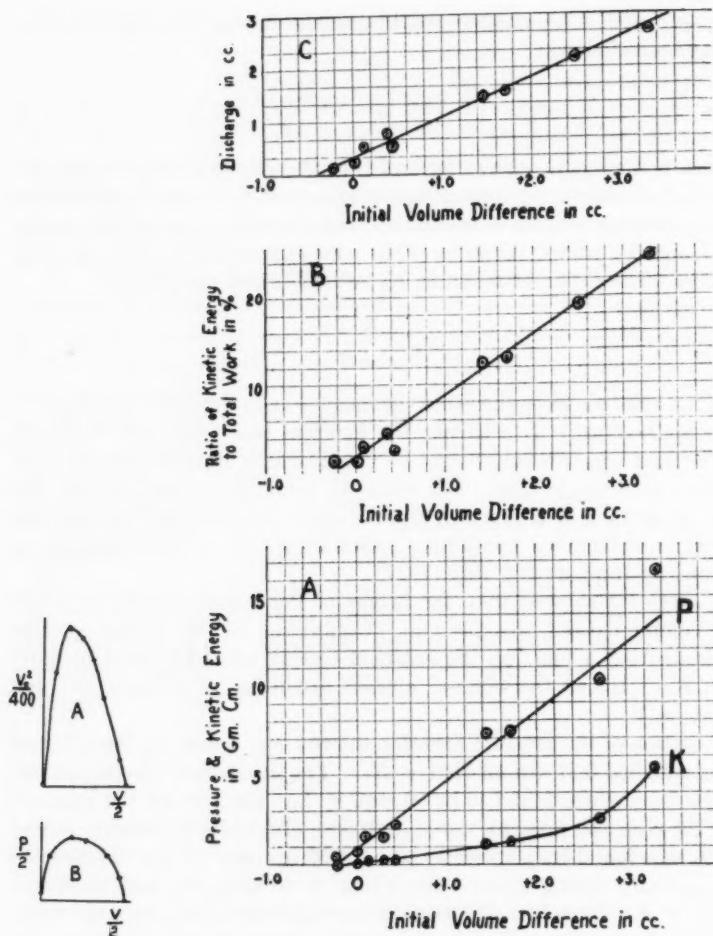
Fig. 1   Fig. 2

Fig. 1. Illustration of the geometric constructions (greatly reduced) used to integrate the kinetic energy (A) and the potential (pressure) energy (B) of the external work from preparation whose curves are shown in figure 2 of a previous report (1930). The ordinates of (A) are expressed in units which are 1/400 of the square of the volume change, the abscissae are in units $\frac{1}{2}$ the volume. The ordinates of (B) are in units $\frac{1}{2}$ the pressure, the abscissae are in units $\frac{1}{2}$ the volume. The area of the original of (A) would therefore be multiplied by 800 to get A_{VM}, and that of (B) by 4 to get A_{pV}.

Fig. 2. Curves showing relationship to the initial volume of systolic discharge (curve C), kinetic energy, K, potential (pressure) energy, P (curve A), and the ratio of kinetic energy to total work (curve B), when the contraction is nearly isotonic.
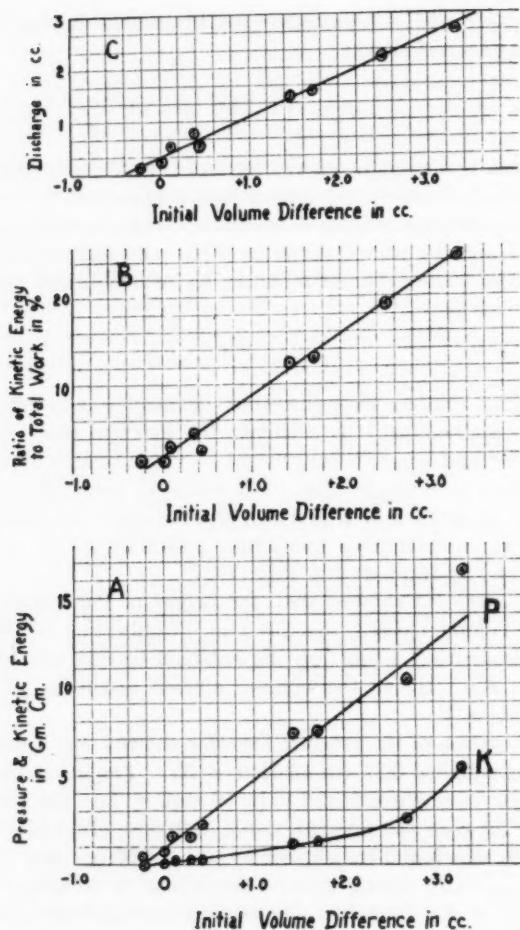
to 34.45 gm-cm., of which the form appearing as kinetic energy constituted 7.12 per cent of the total.

In calculating the external work by integration according to formula (2), it was found that the area beneath the curve (fig. 1 B) constructed by plotting the pressure of the enlarged tracing in millimeters as ordinate against the volume in millimeters during the whole time of ejection, when measured by a planimeter, was 9740 sq. mm. $= A_{pV}$. The pressure energy of the external work, $\int_{T_0}^{T_1} PdV$, according to formula (6) was 39.4 gm-cm.

The area beneath the curve (fig. 1 A) constructed by plotting the square of the change in volume as ordinates against the volume in millimeters for each increment of time during ejection, when measured by a planimeter, was 42,160 sq. mm. $= A_{VM}$. The calibration factor, K_E, according to formula (8), was 3.65 cm./sec., therefore the kinetic energy of the external work, $\int_{T_0}^{T_1} \frac{dMv^2}{2g}$, was 4.3 gm-cm. By formula (2) the external work, W, as determined by integration was equal to 43.7 gm-cm. of which the form appearing as kinetic energy constituted 9.85 per cent of the total.[10]

Similar calculations were made in 60 curves and the differences between the two methods calculated in percentage using the value obtained by integration as 100 per cent. These figures are assembled in summary form in table 1. A minus sign indicates that the value obtained by integration was larger than that given by using the mean value, and a plus sign indicates the reverse. The actual values obtained by integration and the change in initial volume as well as the amount of the systolic ejection are included in this table. At the end of each group of experiments the average error is given and at the bottom of the table the average of these averages.

The error in calculating the external work appearing as pressure energy by using mean pressure is -7 per cent on the average, which falls in line with Frank's computations. However, the range of error is very large, varying in these experiments from $+123$ per cent and $+91.6$ per cent in experiment 28-5 a and 26-1 i, respectively, when the initial volume is small, to -37.8 per cent in experiment 28-11 b. It will thus be seen that the error introduced by using mean pressure may sometimes be very large. The variability of the error is determined by the configuration of the time-curve of the pressure and volume records. An attempt was made to correlate this variability with certain factors. Plots were therefore constructed. Such plots have shown that the error is usually less when the contraction is auxotonic than when it is nearly isotonic, cf. experiments 25-1 a, b and c; 25-3 a, b, and c; 28-3 a, b and c; 28-4 a, b,

[10] HM was $2.01 \times 0.5 = 1.005$ gm-cm. = work in form of energy of position.

TABLE 1

Comparison of external work of turtle heart calculated by integration and by using the arithmetic mean

EXPERIMENT NUMBER	CONDITION OF CONTRACTION	PRESSURE ENERGY		KINETIC ENERGY		RATIO OF KINETIC ENERGY TO TOTAL WORK***		DISCHARGE	INITIAL VOLUME
		Gm.-cm. as calculated by integration	Error in per cent between two methods	Gm.-cm. as calculated by integration	Error in per cent between two methods	Per cent as calculated by integration	Error in per cent between two methods		
								cc.	cc.
18*									
5c	AL'	29.9	−30.1	9.82	−57.2	24.4	−30.6	1.1	
7c	AL	85.3	−21.7	359.5	−40.5	80.8	−18.7	3.7	
7d	AS″	173.5	−28.7	119.0	−68.0	40.8	−30.0	2.2	
8c	AL	55.7	−18.5	53.6	−55.8	49.2	−36.6	1.7	
8d	AS	25.6	−8.2	4.8	−41.0	15.7	−32.8	0.7	
Average			−21.4		−52.5		−29.6		
24									
1a	I†	0.77	+5.7	0.0077	−29.7	1.07	−33.5	0.22	0**
1b	I	2.01	−4.6	0.044	−26.1	2.17	−22.8	0.56	+0.41
1c	I	7.17	−19.6	1.08	−48.8	13.1	−32.6	1.53	+1.68
1c₁	I	10.3	+1.8	2.42	−33.0	19.0	−29.7	2.19	+2.73
1d	I	16.4	−31.0	5.27	−59.5	24.4	−34.0	2.65	+3.33
1e	I	7.18	−13.2	1.01	−60.0	12.3	−50.4	1.49	+1.46
1f	I	3.03	−17.8	0.138	−45.2	4.36	−32.4	0.77	+0.38
1g	I	1.72	−17.7	0.053	−53.3	2.97	−41.6	0.56	+0.13
1h	I	0.40	−21.5	0.0066	−70.0	1.61	−59.4	0.19	−0.22
Average			−10.9		−47.3		−37.4		
25									
1a	I	2.44	−20.5	0.00442	−55.6	0.181	−45.3	0.26	0
1b	AL	2.44	−6.8	0.00656	−65.2	0.268	−60.5	0.26	0
1c	AS	2.44	−5.8	0.00656	−58.4	0.269	−55.7	0.26	+0.14
2d	I	7.52	−23.0	0.397	−51.4	5.02	−35.2	1.10	+1.98
3a	I	11.12	−21.1	0.585	−37.2	4.98	−19.2	1.57	+3.00
3b	AL	13.5	−6.7	0.622	−47.1	4.40	−44.6	1.40	+2.81
3c	AS	14.9	−6.7	0.200	−43.0	1.32	−43.4	1.03	+3.05
Average			−12.9		−51.1		−43.4		
26									
1i	I	0.295	+91.6	0.000308	+100.6	0.104	−9.7	0.09	−1.5
1j	I	1.84	−15.1	0.00626	−46.2	0.338	−36.4	0.32	−0.68
1g	I	4.17	−9.8	0.0443	−39.1	1.05	−32.4	0.59	0
1n	I	5.51	−20.0	0.0396	−52.1	0.714	−38.5	0.66	+0.20
Average			+11.7		−9.2		−29.2		

TABLE 1—*Continued*

EXPERIMENT NUMBER	CONDITION OF CONTRACTION	PRESSURE ENERGY		KINETIC ENERGY		RATIO OF KINETIC ENERGY TO TOTAL WORK***		DISCHARGE	INITIAL VOLUME
		Gim.-cm. as calculated by integration	Error in per cent between two methods	Gim.-cm. as calculated by integration	Error in per cent between two methods	Per cent as calculated by integration	Error in per cent between two methods		
								cc.	cc.
28									
1a2	I	7.9	−13.7	0.0107	−49.6	0.151	−41.7	0.32	0
1a3	I	5.92	−4.2	0.0094	−48.5	0.158	−46.2	0.32	0
1b	AL	4.05	+2.7	0.00714	−57.2	0.176	−58.3	0.26	0
1c	AS	4.54	−2.6	0.0186	−63.9	0.409	−62.9	0.30	+0.07
2a	I	23.1	−8.2	1.08	−39.7	4.47	−33.4	1.28	+1.03
2b	AL	26.2	−11.1	1.37	−48.8	4.98	−41.3	1.34	+1.00
2c	AS	31.5	+14.3	1.36	−25.0	4.14	−33.4	1.29	+1.03
3a	I	39.4	−18.8	4.30	−43.1	9.85	−27.7	2.01	+1.74
3b	AL	33.7	−7.26	2.62	−32.1	7.22	−25.4	1.74	+1.48
3c	AS	42.4	−6.4	2.49	−47.0	5.55	−42.4	1.55	+1.48
4a	I	46.8	−13.9	6.07	−33.7	11.5	−20.8	2.37	+2.36
4b	AL	50.6	−13.4	4.99	−32.3	8.98	−20.3	2.24	+2.40
4c	AS	54.0	−8.9	3.91	−48.7	6.77	−42.0	1.83	+2.16
Average................			−7.0		−43.8		−38 1		
28									
5a	I	0.546	+123.0	0.0102	+35.3	1.83	−38.3	0.17	0
5b	I	9.04	−20.0	0.348	−27.3	3.71	−8.9	0.93	+0.88
5h	I	33.1	−12.1	2.63	−30.1	7.36	−19.3	1.92	+1.54
5i	I	38.6	−11.7	5.00	−23.8	13.0	−19.2	2.78	+1.95
5n	I	20.4	−15.2	3.22	−41.6	13.6	−27.9	2.15	+2.21
5p	I	18.2	−19.3	2.68	−34.8	12.9	−18.6	2.04	+1.32
6b	AS	21.5	+3.7	1.34	−44.0	4.73	−31.3	1.53	+0.76
6c	AS	23.2	+0.4	1.64	−56.5	6.51	−54.3	1.47	+1.73
6d	AS	11.7	−0.4	0.746	−52.0	6.00	−50.3	1.11	+0.23
Average................			+5.4		−30.5		−29.8		
28									
8b	AL	7.5	−11.9	0.826	−45.7	9.94	−35.9	1.28	0
8c	AS	9.12	+1.54	0.445	−42.3	4.65	−42.0	0.96	−0.07
9a	I	5.18	−27.6	0.832	−43.9	13.9	−19.9	1.32	−0.37
9b	AL	6.76	−15.1	0.668	−39.6	8.9	−26.1	1.22	−0.41
9c	AS	6.27	+5.9	0.362	−54.4	5.46	−55.9	0.84	−0.67
10a	I	3.38	−19.2	0.553	−38.6	14.1	−21.3	1.11	−0.49
10b	AL	3.7	−21.1	0.485	−50.5	11.6	−34.5	0.98	−0.45
10c	AS	5.31	−4.7	0.231	−55.4	4.17	−52.0	0.75	−0.41
11a	I	2.36	−13.6	0.284	−35.6	10.8	−23.6	0.84	−0.60

TABLE 1—*Concluded*

EXPERIMENT NUMBER	CONDITION OF CONTRACTION	PRESSURE ENERGY		KINETIC ENERGY		RATIO OF KINETIC ENERGY TO TOTAL WORK***		DISCHARGE	INITIAL VOLUME
		Gm.-cm. as calculated by integration	Error in per cent between two methods	Gm.-cm. as calculated by integration	Error in per cent between two methods	Per cent as calculated by integration	Error in per cent between two methods		
								cc.	cc.
28									
11b	AL	3.55	−37.8	0.210	−42.0	5.59	−6.3	0.80	−0.56
11c	AS	3.04	−1.65	0.141	−44.5	4.43	−42.5	0.59	−0.45
12a	I	3.18	−22.9	0.578	−52.4	15.4	−34.4	1.10	+1.05
12b	AL	4.26	−14.6	0.329	−38.0	7.2	−26.1	0.99	+0.92
12c	AS	5.83	−8.8	0.222	−71.2	3.67	−67.6	0.98	+0.99
Average...............			−13.7		−41.0		−34.8		
Average of averages....			−7.0		−39.3		−33.2		

* This experiment was based on data obtained from tachygrams.

** The initial volume was obtained as a difference in cubic centimeters using 0 as an arbitrary starting point.

′ Auxotonic contraction, connected with tube *H* of figure 1 of a previous report (1930).

″ Auxotonic contraction, connected with tube *G* of figure 1 of a previous report (1930).

† Nearly isotonic contraction, connected with reservoir *E* of figure 1 of a previous report (1930).

*** One-half of the discharge in cubic centimeters will give a close approximation of the total work in gm.-cm. appearing as energy of position. This was omitted in calculating the total external work.

and c; 28-5 b, h and 6 b, c; 28-12 a, b and c. Similarly such plots have shown that the error usually increases when the initial volume increases, cf. 28-1 a, 2 a, 3 a and 4 a, and when fatigue ensues, cf. 24-1 a, b and 1 f, g, although there are many exceptions.

The error in calculating the external work appearing as kinetic energy by using mean velocity is much greater than in the calculation of the pressure energy; the error is on the average −39.3 per cent. The fluctuations in the error are much greater than in the case of the pressure energy, varying from +100.6 per cent in experiment 26-1 i and +35.3 per cent in experiment 28-5 a, in which the initial volume is small, to −71.2 per cent in experiment 28-12 c as the other extreme. Plots showed that the error was usually less in the nearly isotonic contraction than in the auxotonic, cf. 25-3 a, b and c; 28-1 a, b and c; 28-4 a, b and c; 28-5 b, h and 6 b, c; 28-10 a, b and c. An increase in the error occurred when the initial

volume increased, cf. 24-1 a to d; 28-6 b, c and d, and during fatigue, cf. 24-1 a, b and 1 f, g, although there are many exceptions.

The distribution of the external work between the pressure energy and kinetic energy forms also is miscalculated by using mean pressure and mean velocity. This was estimated by determining the ratio between that portion of the external work appearing as kinetic energy and the total. The error in calculating this ratio by using mean pressure and velocity was on the average −33.2 per cent. In individual cases it varied from −6.3 per cent in experiment 28-11 b to −67.6 per cent in experiment 28-12 c. This error was less usually when the heart was contracting under nearly isotonic conditions than when contracting under auxotonic, cf. 28-1 a, b and c; 28-4 a, b and c. An increase in error usually occurred when the initial volume increased, cf. 28-5 b to n, and with fatigue, cf. 24-1 b, c and 1 f, g, although there are many exceptions.

It is obvious from these observations that the use of mean pressure and mean velocity in calculating the external work of the heart introduces a variable error. This operates usually to underestimate both forms of energy but especially the kinetic energy form, so that the distribution of the work of the heart between the two forms of energy is misjudged.

Several other methods of calculating the work of the heart were used in this study, viz. in experiment 19, the mean velocity was determined not only by the method described, but in another set of calculations (not included in the report) by taking the mean of the greatest and smallest velocity during ejection. In the other experiments, besides the method reported in this paper, a set of calculations was made using for the mean pressure the mean of the highest and lowest pressures during ejection. The error introduced by using mean pressure and mean velocity so calculated, was of the order reported and shown in table 1. In other words, the use of mean pressure and mean velocity regardless of how determined, cannot accurately measure the external work of the heart.

PART II. SOME FACTORS MODIFYING THE EXTERNAL WORK OF THE HEART. The ability to control the conditions under which the isolated heart in this preparation contracted lends itself to a study of the factors modifying the external work of the heart.

a. *The systolic discharge and the velocity of ejection.* The amount of the systolic discharge was found to be a function of the initial volume, confirming again Starling's law (1915); the relation between the two being linear as shown in figures 2 C and 3 C. However, the initial volume is not the only factor controlling the discharge. It was found that the conditions under which the heart contracted play a rôle. Thus for a given initial volume the heart discharged less when contracting auxotonically than when contracting nearly isotonically. This difference was found to increase progressively as the initial volume increased, cf. figure 3 C. Fatigue also

was found to affect the systolic discharge; at a given initial volume the fatigued heart discharged less per beat than the non-fatigued one.

The changes in velocity of flow paralleled the changes in systolic discharge, as would be expected with an opening of fixed bore.

b. *The pressure curve during ejection.* The maximum systolic pressure increased progressively with increase in initial volume. At a given initial volume the maximum pressure was greatest in the isometric contraction, and least in the nearly isotonic; the maximum pressure being intermediate in the auxotonic contraction. These observations are in accord with those in the frog by Frank (1898). At a given initial volume fatigue decreased the maximum pressure developed during systole.

It was found that in the auxotonic contraction the pressure at the end of ejection was positive, and the extent of the elevation at this time increased with increase in initial volume and decreased with fatigue. In the nearly isotonic contraction the pressure at the end of ejection was often negative as regards the zero level of the system, the extent of this drop increasing as the initial volume increased. At the same time, the pressure crossed the zero level of the system earlier during the ejection phase. This phenomenon is associated with the rather abrupt decrease in the rate of ejection toward the end of systole, and is due to the momentum of the previously ejected fluid creating a vacuum behind it. It differs in its cause from the drop of pressure in early diastole (Katz, 1930). The pressure at which ejection began became positive in regard to the zero level of the system as the initial volume increased, and a short isometric contraction phase developed because the inertia of the fluid had more influence as the contraction became more abrupt.

c. *External work.* In table 1 are assembled the data showing the correlation of pressure energy, kinetic energy and the ratio of the latter to the total external work, as calculated from formula (2), together with the conditions under which the heart contracted, etc. Analysis showed that

Fig. 3. Curves showing relationship to the initial volume of systolic discharge (curve C), kinetic energy, K, potential (pressure) energy, P (curve A), and the ratio of the kinetic energy to the total work (curve B), when the contraction is nearly isotonic and auxotonic. ● Solid circles and dotted lines are of auxotonic contractions, viz., connected to tube H, figure 1 of previous report (1930); ◑ half solid circles and dash lines are of auxotonic contractions, viz., connected to tube G of figure 1 of previous report (1930); ⊙ open circles with center dot and solid lines are of nearly isotonic contractions, connected with reservoir, E, fig. 1 of previous report (1930).

Fig. 4. A shows the P/V ratios of the fully relaxed and fully contracted heart contracting nearly isotonically and auxotonically. The circles have the same significance as in figure 3. The solid lines express the smooth curves along which the P/V ratios of the fully relaxed, R, and fully contracted heart, C, fall.

B shows the effect of fatigue on the P/V ratios of the fully contracted heart *1 s, 2 s, 3 s* and *4 s*, and of the fully relaxed heart *1 d, 2 d, 3 d* and *4 d*, as compared with the normal curve of these ratios, to wit, R and C.
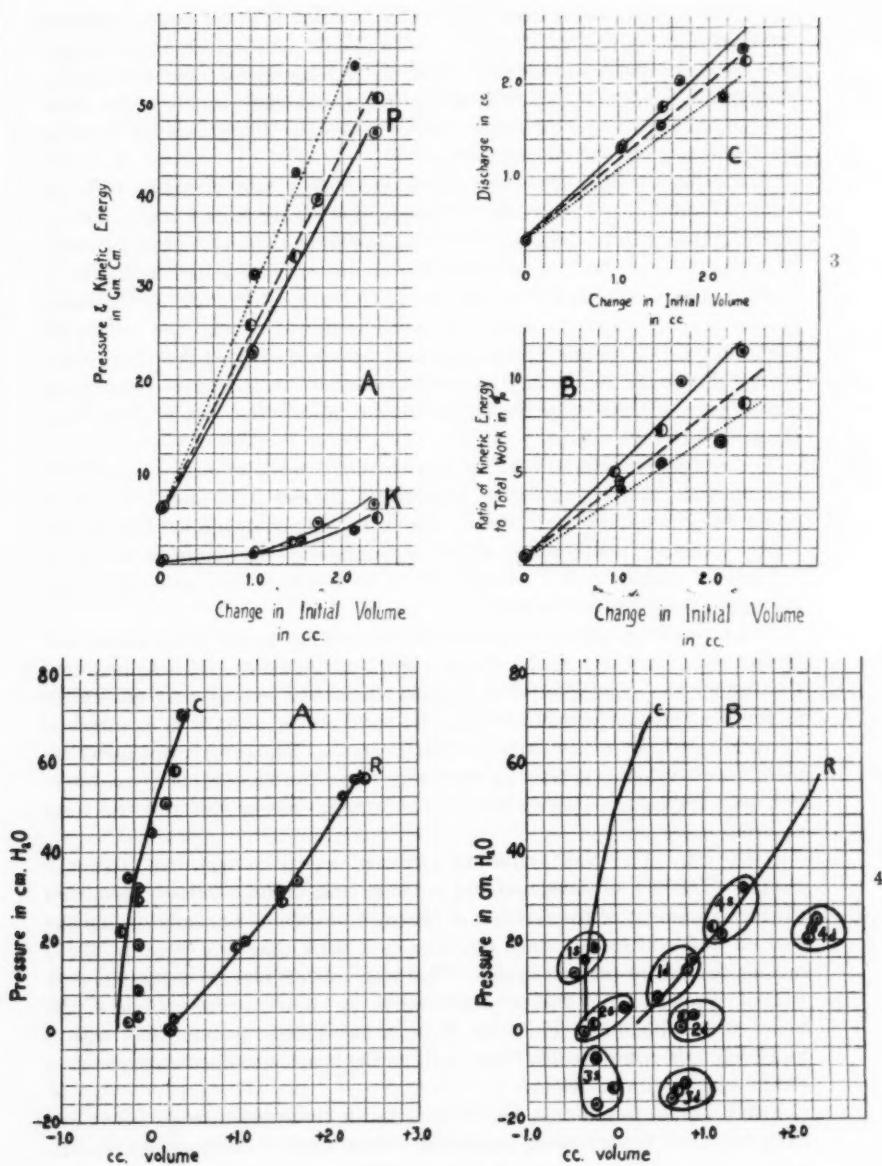
Figs. 3 and 4

the pressure energy increases in the range studied in linear fashion with an increase in initial volume, cf. figures 2 A and 3 A.   At a given initial volume the pressure energy developed is less in the nearly isotonic contraction than in the auxotonic, the difference being greatest when the initial volume is large, cf. figure 3 A.   Fatigue tends to decrease the amount of pressure energy at a given initial volume.

The external work appearing as kinetic energy also increases with the initial volume, but in a geometric progression rather than a linear one, cf. figures 2 A and 3 A.   This is to be expected since the kinetic energy varies with the cube of the rate of ejection.   At the smaller initial volumes the kinetic energy developed in the nearly isotonic contraction is the same, within the experimental error, as that developed during the auxotonic contraction.   At larger initial volumes, however, (fig. 3 A) the kinetic energy developed in the nearly isotonic contraction is greater than in the auxotonic.   Fatigue cuts down the kinetic energy but less in proportion than the pressure energy.

The ratio of kinetic energy to total external work increases in a linear fashion with increase in initial volume, cf. figures 2 B and 3 B.   At a given initial volume it is less in the auxotonic contraction than in the nearly isotonic, a difference which is augmented by increase in initial volume, cf. figure 3 B.   Fatigue tends to increase the ratio of kinetic energy to total external work.

These results emphasize that the kinetic energy in the mammal cannot be ignored in calculating the external work of the heart.   It is to be noted that under the experimental conditions the kinetic energy may constitute as much as 80 per cent of the total external work; often it is as much as 15 to 20 per cent of the total external work.   The error introduced by using mean velocity is serious, especially when the kinetic energy is large, not only because it underestimates the kinetic energy but also the total external work.

PART III. THE VARIATION OF THE P/V RATIO DURING THE CARDIAC CYCLE.   The use of simultaneous pressure and volume curves permitted the establishment of the relation of pressure to volume not only during the relaxed and contracted state but also as a work-diagram (Straub, 1917) during the entire cardiac cycle.   This relationship can be expressed as a ratio P/V, where P is the pressure and V the simultaneous volume of the heart at any instant.   The ratio P/V in any elastic system filled with a relatively non-compressible fluid, such as the heart, becomes an expression of the elasticity coefficient.

Frank (1898) found that the P/V ratio for the relaxed frog's heart was different when it contracted isotonically than when it contracted isometrically.   This, as already reported (Katz, 1930), we failed to find in the turtle heart.   No difference was seen, in eight groups of experiments on five

preparations, in the P/V ratio of the fully relaxed heart, regardless of how
the contraction occurred (cf. fig. 4 A). The lower solid line in this figure,
concave upward, shows the curve of P/V ratios in the fully relaxed heart.
The scatter of the experimentally determined values coincide reasonably
well around this curve. Fatigue, however, produced a marked progressive
lowering of the P/V ratios of the fully relaxed heart; that is, it decreased
the tone of the heart in the sense used by Wiggers (1923). This is illus-
trated in figure 4 B. The lower curve shows the P/V ratios of the fully
relaxed ventricle as determined from figure 4 A. The ratios of P/V are seen
to fall progressively below this curve, viz., 1 d, 2 d, 3 d and 4 d of this
figure, showing that the P/V ratios of the relaxed heart are decreased by
fatigue.

Frank (1898) has pointed out that, at a given initial volume, the maxi-
mum pressure developed is higher when the contraction is isometric than
when it is isotonic, and that the auxotonic contraction is intermediate.
This has been confirmed by several workers (e.g., Katz, Ralli and Cheer,
1928). The question arises whether the P/V ratios of the fully contracted
heart form a single smooth curve, or that the different conditions of con-
traction prevent such a construction. Plots were therefore made of the
P/V ratios of the fully contracted heart under different conditions of
contraction, using the minimum volume as the time of full contraction.[11]
It was found in the eight experiments that the P/V ratios of the fully
contracted heart formed in each experiment a smooth curve which is con-
vex upward, viz., upper curve figure 4 A. The scatter of the experi-
mentally determined values coincide reasonably well around this curve.

The nature of the conditions of contraction determined the change in
pressure and volume but did not cause the ratio to deviate from this
curve. It becomes clear that the reason the heart contracting auxotoni-
cally has a smaller discharge than that contracting nearly isotonically is
the larger pressure the former possesses at the end of systole. Fatigue,
however, lowers the P/V ratio of the fully contracted heart more than the
P/V ratio of the fully relaxed heart. Thus in figure 4 B the P/V ratios
of the fully contracted fatigued heart, 1 s, 2 s, 3 s and 4 s of this figure,
deviate more from the curve of P/V ratios of the fully contracted heart
than do the P/V ratios for the fully relaxed fatigued heart, 1 d, 2 d, 3 d
and 4 d, from the curve of P/V ratios of the fully relaxed heart.

The cyclic variations of the P/V ratio were analyzed by constructing
work-diagrams similar to those used by Straub (1917). Since a single
chambered heart was used whose volume and pressure could be followed
in time, these work-diagrams, figures 5 to 9, are more accurate than his.
The point at which contraction begins is indicated by a short horizontal

[11] In the case of the isometric contraction the maximum pressure was used as the
time of full contraction.

line for each curve, and the cyclic changes go counter-clockwise along the curve.

It was found that the work-diagram of the auxotonic contraction was smaller in area, and its upper peak was displaced to the left as compared with the nearly isotonic contraction, cf. *a* and *i* of figures 5, 6 and 7. On the other hand, the work-diagram of the nearly isotonic contraction dipped down more in the lower left hand corner than the auxotonic. These differences varied in extent at different initial volumes, being largest at large initial volumes when fatigue was absent, e.g., figure 6.
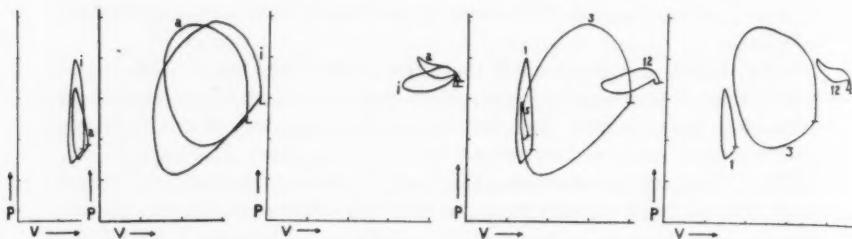

Fig. 5   Fig. 6   Fig. 7   Fig. 8   Fig. 9

Fig. 5. A comparison of the appearance of the work-diagrams of the heart contracting nearly isotonically, *i*, and contracting auxotonically, *a*, at nearly the same initial volume. This and the succeeding work-diagrams are to be read in a counter-clockwise direction, starting at the horizontal line which marks the onset of ejection. The ordinate is pressure *P* and the abscissa is volume *V*.

Fig. 6. A comparison of the appearance of the work-diagrams of the heart contracting nearly isotonically, *i*, and contracting auxotonically, *a*, at nearly the same initial volume. The initial volume is larger than in figure 5.

Fig. 7. A comparison of the appearance of the work-diagrams of the heart contracting nearly isotonically, *i*, and contracting auxotonically, *a*, at nearly the same initial volume. The initial volume is larger than in figure 6 and the heart is fatigued.

Fig. 8. A comparison of the appearance of the work-diagrams of the heart contracting nearly isotonically at different initial volumes, with and without fatigue. The heart when curves 1 and 3 were taken showed no fatigue; when curves 5 and 12 were taken showed fatigue.

Fig. 9. A comparison of the appearance of the work-diagrams of the heart contracting auxotonically at different initial volumes with (12) and without fatigue (1 and 3).

It was found that an increase in initial volume elevated the curve, displaced it to the right and made it more circular, that is, the horizontal diameter increased more than the vertical, cf. 1 and 3 of figures 8 and 9. Fatigue tended to flatten the work-diagrams at a given initial volume, cf. 1 and 5, figure 8; 3 and 12, figures 8 and 9.

Each of the work-diagrams can be divided into four phases of different

steepness and with different types of transitions between them.   The first, during early ejection, consists of an increase in volume associated with a decrease in pressure; the second consists of a decrease in both pressure and volume; the third phase consists of a decrease in pressure with an increase in volume; and the fourth consists of an increase in both volume and pressure.   The first and third phases show how the ventricle initiates emptying and filling, in that the movement of fluid lags behind the change in volume of the ventricle.   The third phase described above shows the drop of pressure during filling which has been alluded to as the suction action of the ventricle (Katz, 1930).

SUMMARY

1. In calculating the external work of the heart it has been the common practice to use mean pressure and mean velocity for this calculation, on the assumption that the error introduced is small.

This was subjected to test in the present research.   An isolated perfused turtle heart preparation was used as it lends itself readily for such an analysis.   Simultaneous pressure and volume curves were recorded by optical means under a variety of conditions, and from these the data were obtained for calculating the work of the heart.   The method of calculating the external work by using mean pressure and velocity is described.   A method was developed of determining the true external work by integration by means of geometric constructions.

It was found that the error in calculating the pressure energy of the external work by using mean pressure was on the average -7 per cent, but varied widely from $+123$ per cent to -37.8 per cent.   The error in calculating the kinetic energy of the external work by using mean velocity was on the average -39.3 per cent and varied from $+100.6$ per cent to -71.2 per cent in individual cases.   The ratio of kinetic energy to total external work was found to have an error, when using mean pressure and velocity, of -33.2 per cent, with a range of -6.3 per cent to -67.6 per cent in individual cases.

It is obvious that the use of mean pressure and mean velocity in calculating the external work of the heart introduces a variable error, which underestimates both forms of energy and the total work; and, in addition, tends to alter the distribution of the work between the two forms so that the per cent appearing as kinetic energy is made less than it really is.   It is not feasible, for this reason, to ignore the kinetic energy in calculating the external work, as has been done in the past.

It was found that the error in determining the two forms of energy and the ratio of kinetic to total work by using mean pressure and velocity tended to increase with augmentation of initial volume, and, at a given initial volume, with fatigue.   This error, at a given initial volume, tended

to be smaller when the contraction was auxotonic than when it was nearly isotonic in the case of the pressure energy; but the reverse was true of this error in the case of the kinetic energy and the ratio of kinetic energy to total work.

2. The systolic discharge, the mean velocity during ejection and the maximum pressure during ejection increased as the initial volume of the heart rose; the relationship between the former two and the initial volume being a linear one. Fatigue reduced the systolic discharge, mean velocity and maximum pressure; at a given initial volume the systolic discharge and mean velocity were less during an auxotonic contraction than during a nearly isotonic one; the reverse was true as regards the maximum pressure during ejection.

In the nearly isotonic contraction it was found that the pressure at the end of ejection was often below the zero level of the system and this was attributed to an effect of the momentum of the ejected fluid. The inertia of the fluid despite the absence of valves led to the occurrence of a short isometric contraction phase at large initial volumes.

The pressure energy and kinetic energy as well as the ratio of the latter to the total work, as determined by integration, increased with an increase in initial volume; the first and last showing a linear relationship to the initial volume while the kinetic energy showed a geometric progression. At a given initial volume, fatigue reduced the pressure and kinetic energy but increased the ratio of the latter to the total work. At a given initial volume the pressure energy was found to be greater and the ratio of the kinetic energy to total work less when the contraction was auxotonic than when the contraction was nearly isotonic, the difference being greatest when the initial volumes were large. No difference between the kinetic energy of the two types of contraction could be made out at small initial volumes, but at large initial volumes the kinetic energy was less in the auxotonic contraction than in the nearly isotonic.

3. It was found that when the heart was not fatigued, the elasticity coefficient—measured as a ratio of pressure over volume—of the fully relaxed heart and of the fully contracted heart formed smooth curves, the former concave and the latter convex upward. The manner in which the heart contracted and relaxed did not cause any deviation from these curves which appear to act as limits. Fatigue, however, tended to decrease the P/V ratios, the effect being more marked on the fully contracted heart.

The cyclic variation of the P/V ratio was analyzed by constructing work-diagrams. It was found that there were four phases in each of these work-diagrams, viz., 1, an increase in pressure and decrease in volume during early ejection; 2, a decrease in both volume and pressure during later ejection; 3, a decrease in pressure and an increase in volume during early

filling—indicating a suction action of the heart; 4, an increase in both pressure and volume later during filling.

An increase in initial volume tended to shift the work-diagram up and to the right, and to make it wider and more nearly circular. Fatigue tended to flatten out the work-diagram. The work-diagram of the auxotonic contraction had the peak of the curve displaced to the left as compared with that of the nearly isotonic contraction, and had less of a tendency to extend into the lower left quadrant.

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THE EFFECTS OF METHYL GUANIDINE SALTS UPON SOME OF THE AUTONOMIC NERVES OF THE DOG[1]

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On account of the violent nature of the symptoms characterizing guanidine tetany considerable effort has been directed toward ascertaining the exact effect of this substance upon the somatic nervous system. It would seem to be of importance to examine also the effect of guanidine compounds upon the autonomic nervous system.

Several investigators have attributed the effects of guanidine compounds upon the heart to action of this substance upon the autonomic nerves supplying this organ. By use of drugs to localize the effects of guanidine compounds, Burns and Watson (1) were led to conclude that these substances have a nicotine-like action upon ganglia and an atropine-like action upon vagus endings. Burns (2) acting upon the assumption that the syndrome of anaphylactic shock is due to the over-activity of the vagus nerves, administered guanidine to shocked animals to prevent or relieve the symptoms, and secured a marked reduction in death rate. The primary cardiac acceleration in frogs following the use of low concentrations of guanidine salts was ascribed by Burns and Watson (3) to the stimulation of accelerator nerves to the heart. The after-slowing was thought to be due to vagus stimulation and to reflex vagus slowing following vasoconstriction. That guanidine acts like a parasympathetic drug is also affirmed by Frank, Stern, and Nothmann (4); and by Frank, Nothmann, and Guttmann (5). Maele and Bulke (6) have observed brief vagal excitation in dogs and rabbits. Sileninkow (7) believes that the effect upon rate and intensity of contraction of intestinal segments of cats is due to action upon Auerbach's plexus.

METHODS AND RESULTS. *1. On the vagus nerve of the dog.* Previous work in the department had shown that methyl guanidine salts have a profound effect upon the ability of the vagus nerve to produce inhibition of the heart in the frog and turtle. This work was undertaken to determine whether or not the same or similar effects would be found to obtain in the dog. The experimental work was done on forty-six dogs under ether anesthesia.

The vagus nerve was dissected out, supplied with a lifting ligature, and cut so that the peripheral end could be stimulated. In most cases the left vagus was used. Injections were made into the left femoral vein by means of a syringe. In administering the anesthetic an ether bottle was used which was so constructed that the valves could be opened wide at the beginning of the experiment and left open, usually without any change during the course of the experiment. Thus changes in the depth of anesthesia were largely eliminated.

Vagus stimulation was by means of the usual laboratory inductorium of the Harvard Apparatus Company. After the strength of stimulation required to give a moderate reduction in blood pressure was found, the secondary coil was fastened and left undisturbed during the rest of the experiment. This procedure was departed from in the case of dog 42. With this dog the aim was to find the minimal effective stimulation at different times during the experiment rather than to keep the stimulation at a constant strength.

It was found by the work on these dogs that methyl guanidine has marked effects upon the vagus nerve of the dog. Doses of 0.025 gram per kilogram of body weight or less often cause an increased effectiveness in inhibiting the heart (as measured by reduction of blood pressure). Larger doses, if injected subcutaneously or slowly intravenously, often have the same effect.

Moderate doses of methyl guanidine sulphate (0.05–0.06 gm. per kgm. of body weight) usually cause a sharp decline of vagus effectiveness followed by a quick recovery to near normal effectiveness, which, in turn, is often followed by a secondary decrease. This is shown graphically in the curve of figure 1. This curve is a composite curve taken from the results obtained on a whole series of dogs. The curve shows the amount that the mean blood pressure could be depressed by stimulating the vagus nerve immediately before the injection of the methyl guanidine (the normal), and at various time intervals after the injections. It will be noted that immediately following the injection the vagus almost completely loses its effectiveness. This "atropine-like" action of the substance is much more lasting in frogs and turtles.

Large doses (0.1 gm. per kgm. of body weight or above) usually cause profound depression of the vagus nerve often accompanied by cardiac irregularities of different kinds. Sometimes these large injections cause slowing of the heart and concomitant block of the vagus nerve. This action appears to be a combination of a pilocarpine-like effect upon the vagus endings and a block somewhere along the vagus pathway, perhaps a nicotine-like effect upon the ganglia. The slowing of the heart caused by these large doses can be quickly and completely stopped by the injection of solutions of atropine. More often these large doses of methyl guanidine cause

cardiac slowing unaccompanied by complete block of the vagus nerve.
These large doses of methyl guanidine are frequently lethal by causing re-
spiratory arrest, the heart continuing to beat long after respiration has
ceased.

Burns and Watson report that injections of calcium lactate restore the
vagus to its normal activity after its effectiveness has been depressed by
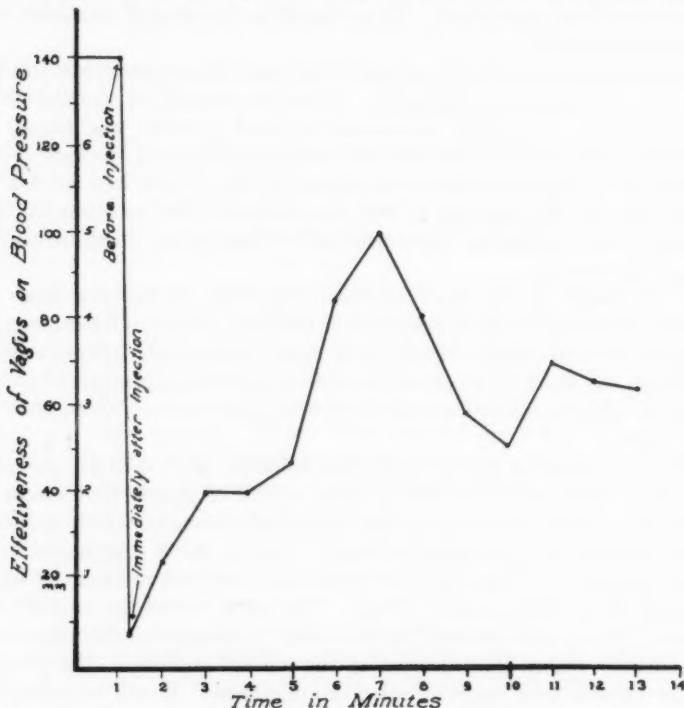
Fig. 1. Composite curve from a series of dogs showing vagus effectiveness in lower-
ing blood pressure before and after injection of 0.06 gram methyl guanidine sulphate
per kilogram body weight.   Vagus irritability is almost completely lost immediately
after the injection.

guanidine.   Since the ability of guanidine salts to do this would appear to
be quite significant with reference to the relationship of guanidine com-
pounds to parathyroid tetany, a group of eight dogs was used in an at-
tempt to verify the findings of Burns and Watson.   While the results
were not striking or conclusive the results when tabulated and compared
with the tabulated results of an equal number of untreated dogs did seem to
indicate some antagonistic action of calcium to the guanidine compound.

The fact that we obtained much less striking results with calcium than did Burns and Watson, we ascribe mostly to the rather quick recovery which occurs in the vagus nerve of the mammal after guanidine injections. We imagine it was this quick, spontaneous recovery which these workers attributed to the effect of calcium.

The effect of physostigmine and of heparmone in removing the methyl guanidine action on the vagus was investigated. Physostigmine was tested because the methyl guanidine effects resembled closely those of atropine and heparmone because others in the laboratory were testing the effectiveness of this substance in combating the pressor effects of guanidine compounds. Neither of these substances showed any antagonistic action to the substance we were testing.

2. On the chorda tympani nerve and the submaxillary gland. Since the methyl guanidine salts we were using had been found to exert a profound influence upon the vagus nerve, it appeared desirable to ascertain whether or not the same or similar effects could be demonstrated upon other autonomic nerves, especially other parasympathetic nerves.

Accordingly we ran a series of experiments upon twenty dogs to determine how the submaxillary gland and the chorda tympani nerve would be affected by injections of these substances. In the first part of the work on the submaxillary gland, the aim was to discover whether methyl guanidine would produce a block in the course of the chorda tympani fibers similar to the block produced in the cardiac inhibitory fibers of the vagus. We quickly discovered, however, that injections of methyl guanidine of 0.05 to 0.06 gram per kilogram of body weight cause a considerable secretion of saliva by the gland. Since the cutting of the chorda tympani and of the cervical sympathetic did not modify this action it was clearly caused by direct peripheral action on the nerve endings or on the gland. A dose of 0.1 gram per kilo of body weight was soon decided upon as best for the work on the salivary gland and was employed from that time on. Doses of this size produce a copious secretion of saliva. Since injections of methyl guanidine cause secretion of saliva it is very difficult to determine conclusively whether or not the parasympathetic fibers are normally irritable to electrical stimulation. In many cases, however, we were able to demonstrate a reduced irritability on the part of the chorda tympani fibers to electrical stimulation. That the flow of saliva was actually due to parasympathetic stimulation was shown by the fact that atropine injections would stop such salivary secretion almost instantaneously. By repeating the injections several times it was possible to produce a complete and lasting block of the chorda tympani fibers to electrical stimulation. On account of the depressing effect of these large doses upon the respiration it was necessary in these cases to give the animal artificial respiration. That the loss of irritability of the parasympathetic fibers in these cases was not due to fa-

tigue produced by long continued stimulation of the chorda tympani, was shown by the control dogs in which there was no loss of irritability over a period of stimulation longer than any of those in which the drug was used.

Again the effect of calcium in removing the guanidine block was tried. It was found that after a complete block of the chorda tympani fibers had been produced, no amount of calcium was effective in removing it.

3. Effect on blood flow through the submaxillary gland.   To determine the effect of the drug used upon the blood flow through the submaxillary gland the vein draining the gland was dissected out; all the other branches of the external jugular vein were tied off; and then the main trunk of this vein was dissected out, cut, and cannulated in such a way that all the blood coming from the gland flowed from the cannula.   In the last experiments heparine was injected into the animal to render the blood incoaguable. All the work was done under ether anesthesia.   In the first of these experiments no precautions were taken to maintain blood pressure at a constant level and in these experiments a rather marked increase of blood flow through the gland invariably resulted.   Since injections of the size we were employing cause a considerable and sustained rise of blood pressure, it was obviously impossible to say whether the increased blood flow was due wholly to this rise of blood pressure or whether vasomotor changes also played a part.   To settle this point it was necessary to keep the blood pressure constant during the course of the experiment.   This was done by means of the blood pressure equalizer described by Jackson (14).

We found that when the blood pressure was kept constant there was no regularity as to the effect on blood flow.   Insofar, then, as the effects of methyl guanidine on the parasympathetic vasodilator fibers of the chorda tympani are concerned, no clear cut results were obtained.   This appears to indicate either that the parasympathetic vasodilator fibers are not stimulated by the drug, or, what appears more probable, that both parasympathetic vasodilator and sympathetic vaso-constrictor fibers are being stimulated to such a degree that the effect of neither constantly predominates.   This conclusion appears all the more likely in view of the apparent stimulating action that this substance exerts upon the sympathetic fibers to the bronchioles.

4. Effect on pancreatic secretion.   From the results obtained in the experiments on salivary secretion it was rather expected that increased pancreatic secretion could be demonstrated following methyl guanidine injections because of the stimulating effect of the drug on vagus fibers.   Accordingly, another series of experiments was run on dogs to determine whether or not such secretion of pancreatic juice following injections of the drug could be demonstrated.

In the first few of these experiments the small upper pancreatic duct was used.   Later the larger lower duct was made use of.   Care was always

taken to disturb the pancreas and especially its blood supply as little as possible. After cannulation of the duct a rubber tube was fitted to the cannula and led out through the wound so that the drops of pancreatic juice could be counted. In almost every case an initial secretion of pancreatic juice was induced by the introduction of 0.5 per cent HCl into the lumen of the duodenum.

The results of this series of experiments show that instead of causing a secretion of pancreatic juice the intravenous injection of methyl guanidine had the opposite effect, i.e., it tended to stop any secretion which was already occurring. After the secretion had been stopped by the methyl guanidine it was usually rather difficult to reinduce it by the introduction of more HCl into the duodenum. Pilocarpine, however, would promptly and effectively reinduce the secretion of pancreatic juice. In one dog, however, even pilocarpine failed to cause a further secretion.

It seems, at first glance, rather strange that this guanidine derivative which had been found to exert profound effects upon parasympathetic nerves elsewhere in the body, should here in the pancreas interfere more with the "secretin" mechanism of secretion than with the vagus mechanism. The explanation for this may be that since guanidine causes vaso-constriction (Maele and Bulcke, 6; Stoland, 8; and Major, 9) and since the pancreas is said to be especially susceptible to changes of blood supply, this substance would tend to affect especially that mechanism that is most directly dependent upon the blood supply, i.e., the secretin mechanism.

Since pilocarpine causes the pancreas to secrete even after rather large doses of methyl guanidine it follows that this substance has not greatly depressed the irritability of that part of the vagus terminations upon which pilocarpine exerts its effects. The actual part of the parasympathetic fibers stimulated by pilocarpine appears to be at or near the myoneural junction (Gaisboeck, 10).

On account of the relationships thought by the Glasgow school of physiologists to exist between the guanidines and parathyroid tetany (11), (12) it is interesting to note that Stoland (13) found a decreased pancreatic secretion following the removal of the parathyroid glands of dogs.

In one dog out of the group a flow of pancreatic juice did follow the injection of methyl guanidine into the femoral vein. This result is so completely at variance with all the other results obtained on this series of dogs that we are at a loss to explain it. Two possibilities suggest themselves, however: There might have been some mechanical obstruction in the duct or cannula early in the experiment that later was overcome, allowing the secretion that had been held back to escape, or the vagus fibers to the pancreas of this dog may have been peculiarly sensitive to the stimulating effect of methyl guanidine.

5. *Effect on the bronchioles.* Another place where autonomic nerve

effects can be readily tested is, of course, the bronchioles. In fact, the bronchioles appear to be exceptionally well adapted for testing the action of substances which, like the guanidines, seem to exert an influence upon both sympathetic and parasympathetic nerves. We employed Jackson's method (14) of recording changes in the size of the bronchioles. This method involves essentially the obtaining of a graphic record of the inflation of the lungs while they are being inflated artificially at a constant pressure. By this method dilatation of the bronchioles is shown on the graph by greater excursions of the marker, and constriction by shorter excursions of the marker. At first we made use of a tambour for recording the lung inflation, but we soon found that when the bronchioles dilated markedly, the rubber covering of the tambour became stretched to the limit of its elasticity and further dilatation of the bronchioles would not be recorded accurately. We then substituted an instrument based on the principle of the spirometer for the tambour and found this recorder satisfactory in every respect.

Fig. 2. Graphic record of bronchial dilatation following injection of 0.1 gram methyl guanidine sulphate per kilogram of body weight.

In some of these experiments there was at times some evidence of parasympathetic stimulation following the methyl guanidine injections. The constriction that occurred in two dogs is indicative of this. The much more pronounced bronchio-dilatation that occurred in another dog following an injection of atropine also indicated that the parasympathetics were being stimulated and were resisting the dilatation of the bronchioles.

However, by far the most striking effect was the bronchio-dilation that occurred at some time shortly following the methyl guanidine injection in every dog except one. This dilatation was, of course, very strong evidence that the sympathetic endings in the bronchioles were being stimulated. To be sure, we recognize the possibility that the bronchio-dilatation might be explained either as the result of sympathetic stimulation, parasympathetic block, or a combination of these two actions. We did not at the time believe that a block of the parasympathetics could explain the results we obtained, because sufficient chloroform had been injected into the vertebral

artery to destroy the activity of the respiratory center, and we felt that if the respiratory center was functionally destroyed, then in all probability the other medullary centers were also destroyed.   With the medullary centers destroyed it seemed hardly possible that a flow of impulses could be occurring over the parasympathetics.   However, to make doubly certain that the effects we were obtaining were really due to sympathetic stimulation, we tried the effect of the injection of the guanidine derivative following an injection of atropine sufficiently large to paralyze the parasympathetic endings.   In each case a well marked dilatation followed the injection of the guanidine.   In figure 2 is shown a graphic record of the bronchio-dilatation obtained in the case of one of the dogs.

The results of our experiments show that methyl guanidine salts exert powerful effects upon certain autonomic nerves.   It is very difficult to say what the physiological significance of the guanidine compounds may be. That the guanidine concentration of the blood is controlled by the parathyroid glands and that after parathyroid removal the guanidine concentration of the blood rises to such a level that tetany is produced seems very doubtful considering the results of many recent investigators of this theory (15, 16, 17, 18, 19, 20).   Some have been inclined to assign to these substances the important function of maintaining muscle tonus (4, 21).   This appears hardly probable because of the toxicity of concentrations sufficient to affect tonus, and further, because the modern conception of tonus as being maintained by a tetanic contraction of small groups of muscle fibers acting successively does not seem to require the participation in the process of any potent chemical like the guanidines.   We have shown that small concentrations of methyl guanidine in the blood stream increase the irritability of some of the parasympathetic fibers.   Since these substances are normally present in very small concentrations (19) they may actually function in maintaining nerve irritability.   It would appear, however, that the probable significance of the body and blood guanidines is that they arise as intermediary metabolites from such substances as choline, creatine, creatinine, or arginine and by diffusion gain entrance into the blood stream without having any true physiological function there.

SUMMARY

1. Methyl guanidine salts have marked effects upon the vagus nerve of the dog.   Small doses, 0.025 gram per kilogram of body weight or less, often cause an increased effectiveness in the cardiac inhibitory action of the vagus when it is stimulated electrically.   Larger doses if injected slowly or subcutaneously often have the same effect.

2. Moderate doses of methyl guanidine (0.05–0.06 gm. per kilo of body weight) usually cause a sharp decrease of vagus effectiveness followed by

a quick recovery to near normal effectiveness, which, in turn, is often followed by a secondary decrease.

3. Large doses (0.1 gm. per kgm. of body weight or more) usually cause profound depression of vagus effectiveness, often accompanied by cardiac irregularities of different kinds. Sometimes these large doses cause slowing of the heart and simultaneous block of the vagus nerve. More often they cause cardiac slowing unaccompanied by complete vagus block.

4. Large doses (0.1 gm. per kilo of body weight or more) are frequently lethal by causing respiratory arrest. This arrest is not due to contraction on the bronchial musculature.

5. Calcium lactate showed a slight value in counteracting the poisoning effect of methyl guanidine on the vagus nerve, but no apparent value in counteracting the block produced in the chorda tympani secretory fibers to the submaxillary gland.

6. The effects upon the vagus nerve of the injections of methyl guanidine seem to be dependent upon several factors: size of dose, the rapidity of introduction into the blood stream, and the previous condition of the vagus.

7. Moderate or large doses of methyl guanidine cause secretion of saliva by the submaxillary gland due to a stimulating effect exerted upon the secretory fibers of the parasympathetic fibers of the chorda tympani nerve.

8. Very large doses of methyl guanidine produce a complete block of the secretory fibers of the chorda tympani fibers.

9. Injections of methyl guanidine into the blood stream give no consistent effects upon the blood supply to the gland.

10. The secretion of the pancreas is slowed or stopped by methyl guanidine injections.

11. Bronchio-dilatation due to sympathetic stimulation follows methyl guanidine injections.

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THE EFFECT OF HIGH FREQUENCY CURRENTS ON THE OXYGEN CONSUMPTION OF FROG MUSCLE

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The experiments described below were planned to determine whether high frequency electric currents of wave length 3 meters and 30 meters have any specific effects on the metabolism of tissues aside from those ascribable to heat. Attempts have been made to answer this question by experiments on whole animals (Nassett, Bishop, and Warren, 1931 and literature there cited). It seemed, however, that the interpretation of the results would be considerably simplified if the experiments could be done in vitro. Having had considerable experience with the frog sartorius muscle for metabolism measurements this was chosen as a standardized test object. The results of over 50 experiments have failed to show any evidence of specific diathermy effects, apart from the heating effect, large enough at least to be easily measurable by this method.

PRELIMINARY EXPERIMENTS. In our first experiments the output of carbon dioxide was measured by observing the changes in the conductivity of barium hydrate contained in the same chamber with the muscle (Fenn, 1928). The muscle was suspended in an atmosphere of oxygen between electrodes which served to conduct the high frequency current. The muscle was further connected by a fine wire passing through a capillary tube to an isometric lever to record any possible contractions. A copper-constantan thermocouple was placed in contact with the muscle and was connected through a suitable cold junction with a galvanometer of such sensitivity that 1.5 cm. represented 1°C. The current had a frequency of 1.08×10^6 per second or 277 meters as measured on a wave meter. Two such experiments were tried with muscles and one with a piece of frog skin and all showed an increased metabolism 5 to 6 times as large as could possibly be accounted for by the temperature change recorded by the galvanometer. The diathermy current caused not the slightest perceptible contraction. The increased carbon dioxide output persisted two to three hours after a heating period of 30 minutes to an hour. The temperature of the muscle rose about 1.7°C. The muscle, however, became non-irritable as a result of the treatment and it soon became evident that this

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was due to the drying out of the muscle, the weight of one pair of sartorius muscles decreasing from 291 mgm. at the beginning of the experiment to 178 mgm. at the end. Such a drying is sufficient to cause loss of irritability and an increased oxygen consumption such as that observed.

Apparatus.   In order to avoid this drying out by the diathermy current the apparatus shown in figure 1A was designed. It consists essentially of a differential volumeter. The muscle is fastened between electrodes in a horizontal position on the stopper of the experimental bottle. A thermocouple is also included to measure the temperature rise in the muscle. The muscle is placed close to the side wall of the tube so that if the apparatus is tipped on its side the Ringer solution in the bottom can be made to flow over the muscle so as to moisten it. Carbon dioxide is absorbed in a small side arm. The rate of absorption of CO_2 in this way is such that
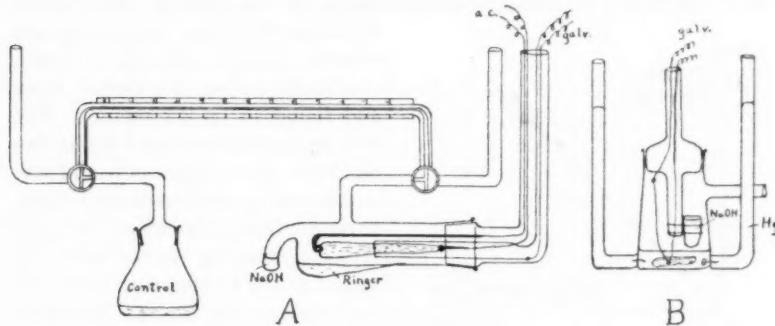

Fig. 1A. Differential volumeter for measurement of oxygen consumption while muscle is heated with diathermy. Apparatus used for series I and II.

Fig. 1B. The experimental bottle of a differential volumeter (apparatus B) used for series III and IV.

approximately 50 per cent of any given amount is absorbed in 5 minutes. Oxygen is measured by the movements of the index drop in the capillary tube.

The diathermy current was conducted to the muscle through the wire electrodes. So far as possible to avoid local heating around the electrodes, cotton, moistened in Ringer's solution, was wrapped around these junctions. The apparatus was continuously shaken mechanically in the water bath except during the diathermy treatment during which time the Ringer's solution was occasionally tipped up onto the muscle.

Another form of bottle was attached to a similar form of respirometer for later experiments (series III and IV) and is illustrated in figure 1B. This permits the muscle to remain in 1 cc. of Ringer's solution during the diathermy treatment. A thermocouple is also immersed in the solution

for the measurement of temperature, the cold junction being located inside the bottle near the stopper. Diathermy was applied through two platinum electrodes, 16 mm. apart, sealed into the vessel and making contact outside with two mercury cups. CO_2 was absorbed in NaOH as indicated.

For the diathermy current we used a powerful vacuum tube oscillator kindly supplied by the General Electric Co. and loaned to us by the Division of Radiology of this School. The wave length was measured as 27 meters by a wave meter. The electric waves were picked up by an antenna connecting with the muscle electrodes. The coupling was so adjusted as to give a rise of temperature in the muscle of 3–8°C. Accurate tuning of the derived circuit was not attempted.

For shorter wave lengths we constructed with two Ux 210 radiotrons a small "push-pull" oscillating circuit similar to one sold by the Central Scientific Co. for demonstrating wireless waves. The wave length was measured on two Lecher wires and found to be 3.2 meters. The heating circuit was coupled with the oscillator by a single loop and was tuned with a condenser until maximum heating of the muscle was obtained. Standing waves were obtained on the Lecher wires both with and without the heating circuit in operation and no change in wave length could be found.
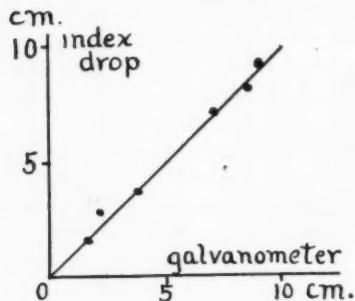
Fig. 2. Calibration curve obtained by heating the muscle with 60 cycle alternating current. The deflection of the index drop is plotted against the deflection of the galvanometer; 1.5 cm. deflection of the galvanometer represents 1°C.

Measurement of the temperature. Early in the experiments our distrust of the galvanometer readings as indications of temperature of the muscle became amply confirmed. Due to capacity effects, for example, the readings would vary with the position of the operator. Fortunately, however, the apparatus was so arranged that we had a very satisfactory A.C. ammeter undisturbed by such effects. As the muscle is warmed by the current, the air in contact with it expands and the index drop is caused to deflect in the capillary tube. This movement could easily be calibrated at the end of the experiment by using a 60 cycle A.C. current in place of the diathermy current and making simultaneous observations of the deflections of the index drop and of the temperature of the muscle, as indicated on the galvanometer. Allowance was made, of course, for the movements of the drop due to the consumption of oxygen. The calibrations with different

muscles varied slightly among themselves but it turned out that approximately 1.5 cm. on the capillary tube of the respirometer corresponded to 1°C., there being a strict proportionality between the two quantities. One such calibration curve is shown in figure 2. Using this method it was found that the average rise in temperature caused by the diathermy in apparatus A was 4.7°C. while the increase as registered on the galvanometer was 8°C. This indicates approximately the amount of the local heating which occurs around the thermocouple. The thermocouple itself consisted of fine wire, lightly paraffined and was buried between two sartorius muscles. The cold junction was located in the stopper of the apparatus and was therefore at the temperature of the water bath itself which was constant in any one experiment to less than 0.01°C. There is no reason to suppose therefore that the temperature of the muscle during the 60 cycle heating was not accurately recorded.
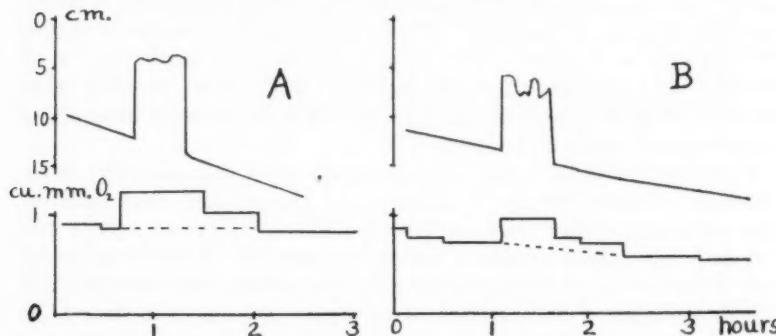
Fig. 3. Graphs of two experiments of series II. Upper graphs represent the positions of the index drop in centimeters and show the deflection obtained during the period of heating. The lower graphs represent the rate of oxygen consumption in cubic millimeters per gram per minute. The rise of temperature of the muscle was 5.6°C. in A and 4.6°C. in B. The calculated values of Q_{10} were 2.7–3.2 in A and 2.2–2.8 in B.

This method of temperature measurement was used with equal success in both forms of apparatus illustrated in figure 1. In apparatus B the calibration was quite constant from one experiment to the next, 1°C. being represented by a deflection of the index drop of 7.6 mm. The current necessary for this degree of heating was approximately equivalent to 16 milliamperes. The actual amount of current which passed through the muscle per degree centigrade rise in temperature was presumably considerably larger in this apparatus than in apparatus A because the rate of cooling to the water bath must have been greater.

EXPERIMENTS. Figure 3 illustrates two heating periods obtained with

muscles in the apparatus of figure 1A.   The rate of oxygen consumption
is always rather high at first and it requires 1 or 2 hours before the base
line is constant enough to permit the diathermy treatment.   The heating
was continued for 20 (occasionally 30) minutes.   By varying the coupling
or tuning between the heating circuit and the oscillator the degree of
heating, as indicated by the index drop, could be maintained fairly con-
stant.   The upper graphs in figure 3 indicate the successive positions of the
index drop and the constancy of the deflection due to the heating is clearly
seen.   From the average deflection so obtained the rise in temperature of
the muscle was obtained.   For this purpose the calibration curve of
figure 2 is necessary.   The rate of oxygen consumption during the dia-
thermy was calculated from readings taken after the heating period was
over and equilibrium was reëstablished in the experimental bottle.   This
usually required 10 minutes.   From the slope of the upper graphs in
figure 2 the rate of oxygen consumption is calculated (neglecting of course
the deflection due to heating).   This rate is plotted in the lower graphs.
There seems to be some excess oxygen consumption persisting for 30
minutes after the heating period is over.   We cannot, however, com-
pletely exclude the possibility that this is merely due to delay in regaining
complete equilibrium in the bottle.

The effect of the diathermy treatment was judged from the value of the
temperature coefficient, Q_{10}, of the increase in metabolism which resulted.
The calculations were made as follows.   The total excess oxygen was
calculated from the beginning of the heating until 9 to 10 minutes after the
end of the heating, when equilibrium had apparently been reëstablished.
On the assumption that all this excess oxygen was taken in during the
actual heating, the excess oxygen was divided by the duration of the heat-
ing to give the excess oxygen rate due to the heating.   This excess oxygen
rate added to the basal rate before heating gives the increased rate of
oxygen consumption.   From the temperature change and the relative
increase in rate of oxygen consumption the value of Q_{10} is calculated assum-
ing that the logarithm of the rate plotted against the temperature gives a
straight line.

The experimental results may be divided into series I, II, III, IV and V.
The corresponding values of Q_{10} are collected in table 1.   As already ex-
plained, the increased oxygen consumption was usually complete in 9 to 10
minutes after the end of the heating period, and the value of Q_{10} was cal-
culated to include this period.   In some experiments, as in figure 3, the
increased oxygen consumption persisted for a somewhat longer period.
In such cases an alternative slightly higher value for Q_{10} was calculated
and is listed in the table.   In still other experiments the oxygen consump-
tion remained permanently increased after the diathermy treatment.   In
such cases the temperature coefficient was calculated to include only the

9 or 10 minutes following the diathermy and the resulting values are followed in the table by a plus sign (+).

The experiments with apparatus A include series I and II with 27 and 3.2 meter waves respectively. The experiments of series I were performed in August, 1930 and those of series II in May, 1931. There seems

TABLE 1

Values of Q_{10}

APPARATUS A		APPARATUS B		
		3.2 meters		Control heating
27 meters	3.2 meters	With shaking	No shaking	
I	II	III	IV	V
$\begin{cases}3.9\\2.4+\end{cases}$	$\begin{cases}2.3\\2.9+\end{cases}$	$\begin{cases}1.6+\\1.0+\end{cases}$	*$\begin{cases}3.7\\5.2\end{cases}$	$\begin{cases}6.8\\2.3+\end{cases}$
$\begin{cases}2.1\text{-}2.5\\3.4\end{cases}$	2.0	$\begin{cases}1.0\\1.3+\end{cases}$	*2.2	$\begin{cases}2.0\\2.6+\end{cases}$
1.9	$\begin{cases}3.2\text{-}26.0\\5.1+\end{cases}$	1.7+	*$\begin{cases}3.8\\2.8\end{cases}$	*$\begin{cases}1.5+\\2.5\end{cases}$
2.7-3.2				
2.2-2.8	$\begin{cases}7.1\text{-}12.3\\10.8+\end{cases}$	*$\begin{cases}1.6\\3.7+\end{cases}$	*$\begin{cases}1.9\\1.8\end{cases}$	*$\begin{cases}2.1\\2.4\end{cases}$
1.5-3.1				
$\begin{cases}1.7\text{-}2.1\\1.6\text{-}2.0\end{cases}$	$\begin{cases}1.8\text{-}2.3\\2.1+\end{cases}$	*$\begin{cases}1.4\\1.9+\end{cases}$	$\begin{cases}7.4\\†4.3\\2.7\end{cases}$	*$\begin{cases}1.0\\2.8\end{cases}$
		* 1.0+	†$\begin{cases}2.4\\2.7\end{cases}$	* 4.0
		*$\begin{cases}2.3+\\2.0\\1.3+\end{cases}$		†$\begin{cases}2.3\\3.9\\1.9\end{cases}$
				† 2.3+

Figures in braces represent successive observations on the same muscle. All the experiments were at 23–24°C. except those at 22°C. indicated by * and those at 14°C. indicated by †. The rise in temperature due to the heating varied from approximately 2°C. to 8°C. in all five series of experiments. Apparatus A and B are illustrated in figure 1. Further details in text.

to be a somewhat greater tendency in the latter series with the shorter wave lengths toward a persistent or prolonged increase in the oxygen consumption. There are also in the latter series some abnormally high values for Q_{10}, notably 7.1 and 10.8 in the fourth experiment, for which no adequate explanation can be offered (see discussion). The apparent slight

difference between the 27 and the 3.2 meter wave lengths might possibly indicate a real difference between these two types of current but it may also be a true temperature difference. All of the experiments of series I and II were performed at temperatures between 23° and 24°C. A further increase of temperature of 3° to 9°C. due to diathermy is sufficient to produce a marked acceleration in the onset of rigor mortis or an injury to the muscle. It is possible that the muscles of series I, taken from summer frogs, were acclimated to a higher temperature so that they would be less injured by this treatment. On account of this difficulty lower temperatures were used in most of the later experiments.

There are, however, certain difficulties involved in the use of apparatus A. There is the possibility of some drying out of the muscle in spite of all precautions and also the possibility of uneven heating of the muscle, the measurement of the temperature of the muscle during diathermy being based upon the assumption that, during the calibration of the muscle with A.C. current, the temperature of the muscle under the thermocouple is representative of the whole muscle. These difficulties are avoided by the use of apparatus B permitting the muscle to remain immersed in Ringer's solution during the diathermy treatment.

The experiments performed with apparatus B may be divided into series III; in which the apparatus was mechanically agitated in the bath throughout the experiment (including the diathermy period), and series IV, in which the apparatus was not agitated. The presence or absence of shaking had no significant effect on the rates of oxygen consumption obtained for the untreated muscle. Taking only the values obtained at 22°C., for example, the average resting rate was 0.99 cu. mm. per gram wet weight per minute with shaking (8 experiments) and 0.92 without shaking (7 experiments). Of the 13 heating periods listed with shaking it will be noticed that all but 4 resulted in a permanent increase in the oxygen consumption. Without shaking none of the 12 heating periods caused such a permanent increase. The shaking consisted in rotating the apparatus backwards and forwards around the capillary tube as an axis about 2 times per second so that the bottles themselves moved through an arc of about 2 cm. in a "sine wave" fashion. To explain why shaking made the diathermy effect apparently so injurious it was suggested that the muscle occasionally was thrown into direct or very close contact with the electrodes, one of which (by an accident) was merely the tip of a wire, so that there was a temporary but very intense local heating effect. No independent evidence could be obtained to confirm this suggestion but it did not seem possible at least to attribute this permanent increase in oxygen consumption to any injurious or stimulating effect of the diathermy current itself, as distinct from its heating effect. If heating with low frequency current is attempted the muscle is, of course, violently stimulated and the rate of oxygen consumption may increase 4 to 8 times.

The values of Q_{10} in series III and IV were all calculated to the end of the 10-minute period following the diathermy, regardless of whether there was a permanent increase in oxygen consumption or not. The average figure so obtained was 1.7 with shaking and 3.4 without. For some reason shaking seemed to delay the onset of the increased metabolism. This was very striking in some experiments in which there seemed to be no purely thermal increase in oxygen consumption ($Q_{10} = 1.0$) all of it being merged with the permanent increase in rate due to injury which succeeded the diathermy treatment.

It seemed possible that this permanent increase in rate might be a purely temperature effect, especially in experiments which were carried out at 24.3°C., which is rather warm for frog muscle. Some control experiments were therefore tried in which the respirometer containing the muscle was transferred from one water bath to another at a higher temperature. By dipping it only momentarily in the warmer water at first and then gradually prolonging the period of immersion this could be accomplished without opening the apparatus to the air or breaking the index drop. The muscle could thus be left at a higher temperature for 20 minutes, as in the diathermy experiments, and then be returned to the original temperature for further readings of the rate of oxygen consumption. A number of experiments of this sort are collected in table 1 as series V. It will be noticed that four of the heating periods were followed by a permanent increase in the oxygen consumption. This was especially true of the experiments at the higher temperatures (24.3°C.) and in muscles after a prolonged survival period. The muscle of the last experiment of series V at 14°C. showed such a permanent increase but had been kept nearly 24 hours in the cold room since dissection. In the first two experiments of series V only the second heating period showed a permanent increase.

A better idea of the actual nature of these experiments may perhaps be obtained by reference to figure 4 in which the rates of oxygen consumption as observed in six different experiments are plotted.

To avoid confusion each experiment is plotted to a different base line the one used being indicated for each experiment by the double arrows at the left. The two upper graphs, a and b, represent two typical experiments of series IV in which the muscle was immersed in Ringer's solution without shaking, with little if any tendency for the increased rate of oxygen consumption to persist longer than can be accounted for by the delay in attaining equilibrium again after the heating period. The next two graphs, experiments c and d, are from two matched muscles from the same frog at 14°C. and belong to series IV and V respectively. The former was heated by diathermy and the latter by removal to a warmer bath. The actual increases in metabolism observed in these muscles for approximately equal intensities and durations of heating were somewhat greater in the dia-

thermy treatment but there is no reason to believe that this small difference is significant.

Graphs e and f (fig. 4) represent two experiments of series III at 22°C. with shaking in which diathermy caused a permanent increase in the
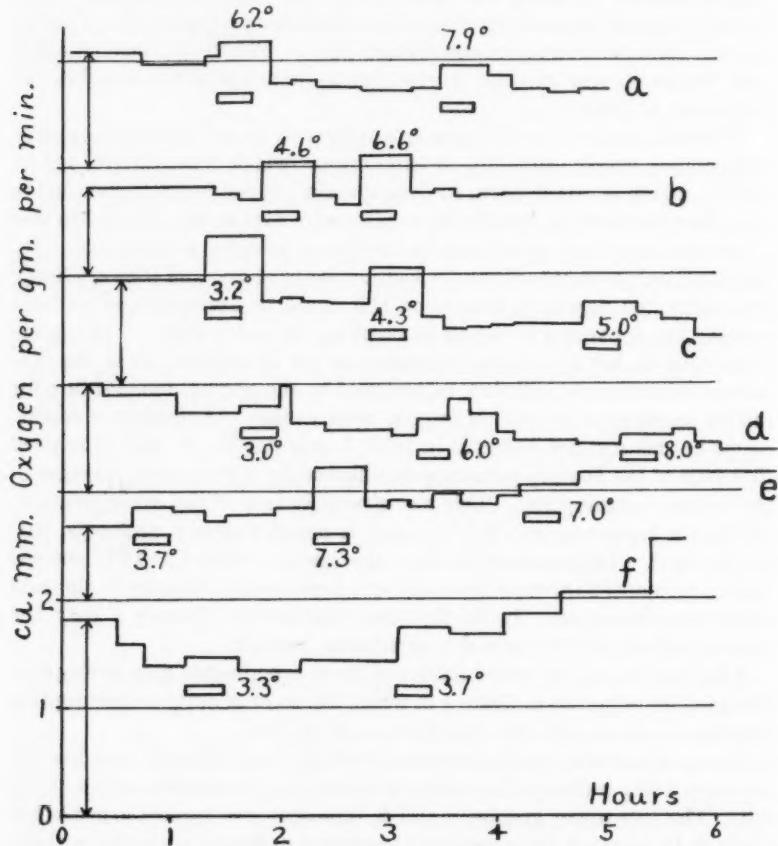
Fig. 4. Graphs of 6 different experiments. Rectangular blocks indicate periods of diathermy. The temperature rise in degrees centigrade is indicated in each case. The calculated values of Q_{10} were: a, 1.9 and 1.8; b, 3.8 and 2.8; c, 7.4 and 4.3 and 2.7; d, 2.3, 3.9, and 1.9; e, 2.3+, 2.0, and 1.3+; f, 1.4 and 1.9+.

oxygen consumption suggesting onset of rigor mortis or irreversible injury.

DISCUSSION. It apppears from these experiments that there is no significant difference between the effects of heat alone (series V) and heat by

diathermy. Moreover the actual values of the temperature coefficient resulting from heating by diathermy are not high enough to indicate any specific diathermy effects. Thus in series I the average Q_{10} is 2.2 to 2.6. Meyerhof (1921, p. 154) gives figures for the resting O_2 consumption of resting frog muscle at temperatures from $0°$ to $22°C$. from which a Q_{10} of 2.56 may be calculated. Previous experiments of our own gave a value of Q_{10} of 2.2 between $22°C$. and $31°C$. A few high values of Q_{10} in series II and IV might be regarded as evidence of a special effect of the shorter wave lengths if it were not for the fact that equally high values occasionally occur in series V. Thus the average Q_{10} in series IV with 3.2 meter diathermy is 3.4 ± 1.6 while the average from series V with heat only was 2.9 ± 1.6 (standard deviations). (Experiments showing a permanent increase in metabolism were omitted.) Such large deviations make the difference between 3.4 and 2.9 insignificant.

It may be concluded that 3.2 meter waves have no effect upon metabolism, other than a temperature effect, which is large enough to be appreciable by this method. It is possible that a much larger series of experiments if treated statistically might show a small diathermy effect. In looking for such an effect it would be better not to use highly irritable muscle tissue, but rather a tissue like kidney where the metabolic rate is higher and less dependent upon imperceptible functional changes. Weiss (1922) for example has shown in muscles that the prolonged passage of a sub-threshold direct current, though without any stimulating effect, could nevertheless cause increased phosphate liberation, earlier incidence of potassium injury, decreased excitability in all parts of the muscle and hence presumably increased oxygen consumption. Diathermy might have an effect of this sort which would be quite independent of any effect upon the oxidative processes themselves. Such an effect might possibly explain the occasional large values of Q_{10} which we have recorded.

The question should perhaps be raised whether muscles treated with diathermy while immersed in Ringer's solution receive as much current as the solution surrounding them. From the data of Sapegno (1931) it is found that the conductivity of a frog muscle at a frequency of 10^7 cycles per second is equivalent to that of a 0.685 per cent solution of NaCl which is practically the same as Ringer's solution. Hence the current and the heating effects should be not appreciably different in the muscle and in the solution. The muscle might be expected to be slightly warmer than the solution because of the fact that it cools by conduction only and not by convection also like the solution. In thin sartorius muscles this difference must be very small.

SUMMARY

Measurements were made of the oxygen consumption of frog sartorius muscles during treatment with alternating currents of approximately 100 million and ten million cycles per second. No special effect could be observed which was not attributable to the heating effect of the current.

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CHANGES IN BLOOD FAT PRODUCED BY FASTING, PHLORHIZIN AND PANCREATECTOMY

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Much information on the mechanisms involved in the metabolism of fat has been gained by determinations of the fat content of organs. Rosenfeld (1913) concluded that under certain conditions, for example, phosphorus or phlorhizin poisoning, fat left the depots and was absorbed and stored by liver and heart, and later work has shown that the muscle (Terroine and Weill, 1913) and kidney (Imrie, 1914) may also store fat. Moreover, there is evidence from studies of gaseous exchanges that striated muscle (Takane, 1926; Himwich and Castle, 1927; Himwich and Rose, 1929; Richardson, Shorr and Loebel, 1930), heart (Starling and Evans, 1914; Peserico, 1925), kidney (Shorr, Loebel and Richardson, 1930; Dickens and Simer, 1930), and testicle (Himwich and Nahum, 1929a; Shorr, Loebel and Richardson, 1930; Dickens and Simer, 1930) may oxidize fat. Brain alone of all mammalian tissue examined has a respiratory quotient fixed at unity (Himwich and Nahum, 1929b; Dickens and Simer, 1930).

Determinations of fat changes of muscle have not led to consistent results. Leathes (1906) and Winfield (1915) found no decrease in the fat content of muscle after tetanization, although Palazzolo (1913) did. Lafon (1913) drew samples of arterial and venous blood of resting muscle and observed less fat in the venous sample. Moreover, there was a still greater removal of fat from the blood passing through the exercising muscle when compared with the fat content of the arterial blood drawn previously during rest. On the other hand, Grigaut and Yovanovitch (1925) noted that the muscles of resting dogs added fat to the blood. It seemed possible that more light could be thrown upon the relation of the various organs to the metabolism of fat by determinations of the fat content of samples of afferent and efferent blood.

METHOD. In the analysis of blood for fat a modification (Himwich, Friedman and Spiers, 1931) of the method of Stewart and White (1925) was used. The procedure consisted in the extraction of fat from plasma of whole blood with a solution of alcohol and ether, saponification of the fatty acids by addition of alkali, the freeing of the fatty acids by the addition of an amount of acid exactly equal to that of the alkali, and finally titration of the free fatty acids by dilute base. This procedure was done in triplicate on 1 cc. samples of whole blood or plasma. The error of the method was ±11 mgm. per cent. Differences of 40 mgm. per cent or more in the fat content of two samples were considered significant. The experimental animals were prepared in 3 ways. Eight normal dogs were fasted from 2 to 3 days, 17 were phlorhizinized of which 13 were studied when the D/N ratio had become constant and the others later on the fifth to seventh day of diabetes; 24 dogs were depancreatized and ex-

TABLE 1

Fat content of whole blood—milligrams per cent

NUMBER	HEPATIC VEIN	PORTAL VEIN	ARTERIAL	VENOUS	CONDITION
1	451	749	390	197	Fasted (3 days)
2	282	350	185	345	Fasted (3 days)
3	365	556	354	461	Fasted (3 days)
4	529	547	825	654	Phlorhizinized (3 days)
5	466	538	574	404	Phlorhizinized (4 days)
6	592	618	879	789	Phlorhizinized (3 days)
7	1,379	1,310	1,345	1,198	Depancreatized (4 days)
8	1,488	1,337	1,408	1,221	Depancreatized (4 days)
9	1,251	1,017	1,156	1,039	Depancreatized (3 days)

amined at the following intervals after the operation, 2 at 6 hours, 6 at 12 hours, 5 at 16 hours, 2 at 24 hours, and 9 after 3 or 4 days. The blood was drawn from the femoral artery and femoral, portal, and hepatic veins, under amytal anesthesia during rest and in 3 experiments on depancreatized dogs in exercise produced by electrical stimulation of the lower extremities. In addition, a study was made of the fat exchanges of the kidney of another series of dogs by analyses of the fat content of samples of arterial and venous blood drawn simultaneously. Sixteen observations were made on the kidneys of normal dogs, 18 on phlorhizinized, and 9 on depancreatized animals.

RESULTS. Table 1 presents typical results on fasted, phlorhizinized, and depancreatized dogs. Summaries of 94 observations of muscle, organs drained by the portal vein, liver, and kidney, are found in table 2. It may be seen by comparison of values of the arterial and venous blood in table 1, that muscles of normal dogs fasted from 2 to 3 days either added

or removed fat from the blood. Table 2 shows that the muscles of fasted dogs liberated fat into the blood stream on 5 occasions and removed it on 3 others. On the other hand (table 1), the muscles of phlorhizinized and depancreatized dogs absorbed blood fat more consistently. This occurred (table 2) in 10 of 13 significant observations of phlorhizinized dogs and 12 of 15 depancreatized animals. Table 1 discloses further that in the fasted dogs the region drained by the portal vein was liberating fat, since portal blood contained more fat than the arterial, and that this liberation was not so constant in the case of the phlorhizinized and depancreatized animals. From an examination of the tables and on the basis of Burton-Opitz' (1911) ratio that 0.3 of the afferent blood of the liver comes from the hepatic artery and 0.7 from the portal vein, it can be calculated that the

TABLE 2

Changes in fat content of blood on passage through organs

CONDITION OF ANIMAL	MUSCLE			ORGANS DRAINED BY PORTAL VEIN			LIVER			KIDNEY		
	Added*	No change**	Removed‡	Added*	No change**	Removed‡	Added*	No change**	Removed‡	Added*	No change**	Removed‡
Fasted.................	5	0	3	7	1	1	1	3	4	3	9	5
Phlorhizinized..........	3	6	10	8	4	7	4	5	10	0	8	12
Depancreatized.........	3	6	12	5	4	9	10	6	2	2	1	6

* Number of observations in which there was an increase of 40 mgm. per cent or more of fat.

** Number of observations in which the changes were within ± 20 mgm. per cent of fat.

‡ Number of observations in which there was an absorption of at least 40 mgm. per cent of fat.

liver of starved and phlorhizinized dogs usually take fat from the blood passing through that organ. In 14 significant observations of phlorhizinized dogs the liver removed fat 10 times. In sharp contrast are the results of the depancreatized animals whose liver added fat to the blood in 10 of 12 significant observations.

In an effort to determine the cause of the different action of the liver, in the phlorhizinized and depancreatized dogs, studies were conducted at varying periods after the removal of the pancreas. Typical experiments are given in table 3 and summaries in table 4. By the sixth post-operative hour the blood fat concentration had hardly risen above the normal but at 16 hours and undoubtedly at 24 hours the liver was adding fat to the blood as in the 3 and 4 day experiments shown in table 2. Only for a short period 12 to 14 hours after pancreatectomy were a larger number of

observations found in which the liver was removing fat from the blood passing through it. In the phlorhizinized dogs studied at a later period (5 to 7 days) the reverse effect to that shown in table 2 was found in that the liver was liberating fat in 7 observations, twice there was no change, and only once was fat removed by that organ.

In many instances there was a significant decrease in the total fats in the venous renal blood as compared with the total fats of the arterial blood. This occurred in 5 of 8 significant observations on the kidney of fasted dogs (table 2). Of 18 pairs of samples of renal blood of phlorhizinized dogs, none showed an increase in the fats of the circulating blood

TABLE 3

· *Fat content of plasma of depancreatized dogs—milligrams per cent*

NUMBER	HOURS AFTER PANCREATECTOMY	HEPATIC VEIN	PORTAL VEIN	ARTERIAL
1	6	785	734	778
2	12	1,172	1,387	1,499
3	16	2,895	2,281	2,593
4	24	3,281	2,999	3,116

TABLE 4

Changes in fat content of blood on passage through the liver of depancreatized dogs

POST-OPERATIVE	ADDED*	NO CHANGE**	REMOVED‡
hours			
6	3	1	1
12	7	2	10
16	9	1	4
24	4	0	0

*
** } as in table 2.
‡

following its passage through the kidney. Twelve disclosed a definite decrease which varied from 49 milligrams per cent to 128 milligrams per cent. The results on the kidney of the depancreatized dogs were not so consistent, since fat was removed in but 6 of 8 significant observations. However, in each of these 6 observations the amount of fat that was removed was large and varied from 79 mgm. per cent to 153 mgm. per cent.

DISCUSSION.   From the above results it must be apparent that there are some factors making for increased blood fat and others diminishing blood fat.   Increases of blood fat may be produced by mobilization of the fat depots and a new formation of fat, decreases are due to an absorption of fat by the tissues where it may be either oxidized or stored.

Muscle. The changes in the fat content of the blood passing through muscle reveal the fact that both processes just mentioned are operative in that organ. There may be a mobilization of the fat depots of the lower extremities with the resulting increase in the venous blood, as in the fasted dogs (tables 1 and 2). On the other hand, fat may be removed by muscle of fasted animals, and particularly so in the phlorhizinized and depancreatized dogs. In the diabetic animals the mobilization of the fat depots had been in progress for some time and therefore was presumably going on at a lessened rate, thus permitting the removal of fat by the muscle to become apparent. These observations are in accord with those of Terroine and Weill (1913); Mayer and Schaeffer (1913), and Terroine (1914–15) who noted fat storage in muscle. While the removal of fat does not prove the oxidation of that foodstuff by muscle it certainly permits such a process to take place. This evidence completes that obtained by a study of the gaseous exchanges of muscle which has indicated the oxidation of fat by muscle both in situ (Himwich and Castle, 1927; Himwich and Rose, 1929) and in vitro (Takane, 1926; Richardson, Shorr and Loebel, 1930).

Region drained by the portal vein. The effects of mobilization of the mesenteric fat depots in the region drained by the portal system are apparent in the dogs fasted but a short period. On the other hand, in the phlorhizinized and depancreatized dogs, starved for a longer time, the factors making for utilization are more prominent, for fat is liberated less frequently.

Kidney. In the kidney mobilization of fat is not predominant over usage. The results of the normal phlorhizinized and depancreatized dogs indicate that fat may be absorbed as the blood passes through the kidney, and this was more consistently the case in the phlorhizinized and depancreatized animals in which the carbohydrate oxidations were diminished.

The average fat absorption per 100 cc. of blood passing through the kidney in 18 observations of phlorhizinized dogs was 51 mgm. and in 9 observations of depancreatized dogs, 66 mgm. This fat must have been oxidized, excreted or stored. However, the oxygen consumption of the kidney could account for the combustion of but a small proportion of the fat absorbed. Since the excretion of fat by the kidney is doubtful (Peters and Van Slyke, 1931) it is more probable that fat is stored. It is true that Rosenfeld (1903) denied the storage of fat in kidney, but Lebedeff (1883) and Sandmeyer (1892) did find accumulations of fat in that organ. Imrie (1914), in a study of human kidney, observed a high fat content in a case of diabetes with lipemia. Furthermore, such a conclusion is in accordance with that of Ray, McDermott and Lusk (1899–1900) that the tissues of diabetic animals absorb more fat than can be oxidized. As stated by Richardson and Mason (1923) the tissues of diabetic patients soak up ingested fat like a sponge.

Liver. The results of the liver experiments are quite decisive. The livers of starved dogs frequently removed fat from the blood passing through them. This phenomenon occurred more often in the livers of phlorhizinized dogs and is in accordance with previous knowledge, since it is well known that in such conditions the depot fat is mobilized and appears in the liver in increased amounts (Rosenfeld, 1903). In the later stages of phlorhizin diabetes (5–7 days) the fat stored in the liver may pass into the hepatic venous blood to be utilized in other parts of the body.

The livers of depancreatized dogs, far from absorbing fat, liberate that substance into the blood stream. Indeed, the level of blood fat in the depancreatized dog is usually higher than that of the phlorhizinized animals (table 1). The observations of this interesting fact were made on whole blood samples. In order to check these experiments, eight additional determinations were made on the plasma of 5 depancreatized dogs and the results were corrected for changes in concentration of the plasma. In 6 of the 8 observations the liver poured fat into the blood.

Apparently the factor which determines the action of the liver is the rate of mobilization of the fat depots. In the phlorhizinized dogs the process is slow. Rarely is the level of blood fat raised much above that of the normal fasting animal and the liver gains in fat for a number of days until the balance is shifted in the opposite direction. After pancreatectomy there comes a sudden almost explosive mobilization of the fat depots in approximately 12 hours (between the 6th and 16th hours), and thereafter fat is gradually released for the use of the other organs of the body.

The time coincidence suggests a correlation between the early mobilization of fat and the carbohydrate changes in depancreatized dogs noted by Chambers and Coryllos (1926). They found a gradual rise in blood sugar culminating in a glycosuria about the 9th to 12th hour after operation, and at the same time a marked increase in nitrogen elimination. The peak of excretion of glucose and nitrogen occurred about the 18th to 21st hours. It is evident from these results that radical changes in the metabolism of the depancreatized dog occur within 24 hours after the excision of the organ.

SUMMARY AND CONCLUSIONS

Observations, 144 in number, were made on the blood of the organs of fasted, phlorhizinized and depancreatized dogs. Most striking were the results obtained on the liver, indicating that that organ removed fat from the blood of fasting animals. The liver of phlorhizinized dogs removed fat from the blood until the 5th day when the accumulated fat was liberated. The liver of depancreatized dogs on the other hand added fat to the blood except for a short period approximately 12 hours post-operative,

when that foodstuff was absorbed. It is suggested that these phenomena are due to the more rapid mobilization of the fat depots of the depancreatized animals.

An increased concentration of fat in the portal and femoral vein which is apt to appear in the dogs fasted 2 or 3 days, indicates a mobilization of the fat depots. On the other hand, observations obtained after more prolonged fasting in the phlorhizinized and depancreatized dogs more often revealed a removal of fat from the blood passing muscle and viscera. The kidney of phlorhizinized or depancreatized dogs absorbed fat from the blood and in amounts too great for oxidation, possibly indicating an excretion, but more probably a storage, of fat in that organ.

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CEREBROSPINAL FLUID IN NORMAL DOGS[1]

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Since Cotugno (1) first described cerebrospinal fluid in dogs, many investigations have been made on the cerebrospinal fluid of this species. However, the normal values of the fluid, such as amount obtainable, pressure and cell count, have received only passing mention in the literature.

In the course of our work on experimental meningitis in dogs, we found it necessary to determine the normal values of cerebrospinal fluid, as well as the comparative values of normal fluid from the cistern and from the lumbar subarachnoid space. In order to obtain an approximate evaluation of the normal cerebrospinal fluid, a series of dogs in whom clear cerebrospinal fluid was obtained on the first attempt were used for the statistical computation of norms and averages.

An attempt was made with each puncture to measure the pressure of the cerebrospinal fluid before the loss of any fluid, to measure the amount of fluid withdrawn, to test it for the presence of globulin increase by the Pandy and Ross-Jones tests and to count the cells. In addition, the pressure was taken in a series of dogs before and after the removal of a measured amount of cerebrospinal fluid, and Ayala's "Quotient Rachidien" calculated from these figures.

All of our observations were made on cerebrospinal fluid removed from dogs under the effect of morphine-ether anesthesia, which factor unquestionably causes some change in the fluid. For instance, morphine causes a fall in the cerebrospinal fluid pressure, an observation that has been made previously by Plaut (2).

Whether there is a corresponding change in the cell count we do not know. It must therefore be remembered that the term "normal" postulates the experimental conditions under which the work was done.

General characteristics. In most respects the cerebrospinal fluid of the dog resembles that of the human subject. It is clear, colorless and limpid, and the usual tests for increased protein are negative. Like the human fluid also, it is devoid of any bactericidal activity.

Amount obtainable. The amount of cerebrospinal fluid obtainable from the subarachnoid space of a dog before the initial pressure falls to zero, or to its minimum, varies more or less directly with the size of the dog. Ordinarily, in a dog 7 to 10 kilograms in weight, cistern puncture will yield from 1 to 5 cc. and lumbar puncture from 0.5 to 3 cc. of fluid. Table 1

TABLE 1

Summary of observations on amount obtained, pressure and cell count of cerebrospinal fluid in normal dogs*

	CISTERN				LUMBAR			
	Number of observations	Range	Mean ± probable error	Standard deviation	Number of observations	Range	Mean ± probable error	Standard deviation
Amount in cc...........	45	1 to 8	3.4±0.17	1.7	27	0.5 to 5	2.2±0.22	1.7
Pressure in cm. of water..	37	3 to 23	14.3±0.53	4.8	22	5 to 18	12.3±0.53	3.7
Number of cells.........	58	0 to 10	2.2±0.20	2.2	39	0 to 10	4.0±0.30	2.8

* The average weight of the dogs was about 9 kilograms.
All computations were done by the usual standard statistical methods.

TABLE 2

Reliability of differences between means of cistern and lumbar observations

	DIFFERENCE BETWEEN CISTERN AND LUMBAR MEANS $M_c - M_l = D_m$	PROBABLE ERROR OF THIS DIFFERENCE = $P.E._d = \sqrt{(P.E._c)^2 + (P.E._l)^{2*}}$	RATIO $D_m : P.E._d$	PROBABILITY OR RELIABILITY
Amount in cc.................	1.2	0.28	4.3	267:1†
Pressure in cm. of water......	2.0	0.75	2.7	14:1‡
Cells.........................	1.8	0.36	5.0	1341:1†

* This formula for the probable error of a difference is not strictly correct for the average cell count where the same dog, in twenty instances, was used for both determinations. However, the more correct formula, which is $P.E._d = \sqrt{(P.E._c)^2 + (P.E._l)^2 - 2r_{cl} (P.E._c) (P.E._l)}$, would only give a smaller value to $P.E._d$, and thus a still larger ratio of $D_m : P.E._d$.

† Certainly significant.
‡ Questionable significance.

shows the results of our observations in seventy-two dogs. In forty-five, cistern punctures were done and an average of 3.4 ± 0.17 cc. was obtained, with a range of 1 to 8 cc. In twenty-seven, lumbar punctures were done and the average was 2.2 ± 0.22, with a range of 0.5 to 5 cc. Thus an average of 1.2 cc. more was obtained from cistern than from lumbar puncture. In order to determine whether this 1.2 cc. difference was really due

to a difference in the site of puncture rather than to a mere "chance" distribution of observations, the probable error of this difference was determined and found to be 0.28 (table 2). Since a difference must be at least three times as large as its probable error to be considered significant (or reliable) and since this average is 4.3 times its probable error, we may conclude that this difference of 1.2 cc. represents a real difference due to the site of withdrawal. Another way of stating this is that the chances are more than 267 to 1 that the results obtained were not due to "chance."

Pressure. The cerebrospinal fluid pressure is rather variable, depending upon numerous factors, such as the blood pressure, the depth of anesthesia, the type of anesthesia, the site of puncture, etc. It varies more or less directly with the size of the dog, although there are many exceptions to this rule. The results of other observers of the cerebrospinal fluid pressure in dogs have been reviewed by Weed (3).

As stated above, in our work dogs were narcotized with morphine ($\frac{1}{4}-\frac{3}{4}$ gr.) and then about thirty or sixty minutes later were anesthetized with ether. In fifty-nine dogs pressure readings were taken with a Claude tambour manometer with the animal in the lateral recumbent position. In each case, pressure over the jugular vein was made and a rise in pressure observed, indicating that the canal had been properly entered and that there was no block. In thirty-seven dogs, determinations of cistern pressure were made, the average being 14.3 ± 0.53 cm. of water and the range 3 to 23 cm. of water; in the remaining twenty-two animals, determinations of lumbar pressure were made, the average being 12.3 ± 0.53 cm. of water and the range 5 to 18 cm. (table 1).

There is a difference of 2.0 cm. of water between the averages for cistern and lumbar pressure, the former being the greater. Since, theoretically, with the animal in the horizontal position, the cistern and lumbar pressure should be alike, and since Ayer and Solomon (4) have found that in the horizontal human subject they are identical, we checked the pressure at the cistern and at the lumbar subarachnoid space simultaneously in a series of eight dogs and found them to be the same within the limits of 1 cm. of water. In table 2 it is seen that the difference of 2.0 cm. between cistern and lumbar pressure is only 2.7 times its probable error. In other words, this difference of 2.0 cm. may be due (one in fourteen chances) to merely a chance distribution of an insufficient number of observations and not due to a true local difference.

"Quotient Rachidien" of Ayala. Incidental to our routine observation of the initial pressure of the cerebrospinal fluid and the measurement of the amount withdrawn, we made eighteen measurements in eleven dogs of the final lumbar pressure after drawing off a measured amount of cerebrospinal flluid. From these three observations—namely, the initial pressure (I), the final pressure (F), and the amount of cerebrospinal fluid

withdrawn (A), the "Quotient Rachidien" of Ayala (5) was calculated from the equation: $Q_R = \dfrac{F.A}{I}$. This quotient which is a measure of the *rate of fall* of pressure and which is roughly proportional to the amount of cerebrospinal fluid present in the system is not constant, and may vary in the same animal. However, according to Ayala and Balduzzi (6), in the human subject, quotients above 7 speak for an inflammatory meningitis and below 5 for brain tumor, the normal range being between 5 and 7.

In our work the quotients ranged from 0.33 to 1.37, half of them falling between 0.46 and 0.83 (the interquartile range). The average Q_R was 0.70 ± 0.04, and the standard deviation 0.27. The significance of higher or lower values of Ayala's quotient is outside the province of this paper.

Cell count. Cell counts on all cerebrospinal fluids were done in the usual manner, only macroscopically clear fluids being utilized. Fluids with no grossly visible evidence of blood, which on microscopic examination contained over 500 erythrocytes per cubic millimeter, were discarded. Since the presence of 500 erythrocytes per cubic millimeter in a fluid would mean the presence of about one leucocyte due to the blood admixture, white cell counts on such fluids were deemed as being correct within the limits of error.

Ninety-seven cerebrospinal fluids were obtained from seventy-seven dogs, i.e., from twenty of these animals, both cistern and lumbar fluids were obtained simultaneously. The number of cells in fifty-eight cistern fluids varied from 0 to 10 per cmm., with an average of 2.2 ± 0.20. In thirty-nine lumbar fluids the range was from 0 to 10 with an average of 4.0 ± 0.30 cells per cmm. We thus found a higher cell count than was observed by Flateau and Handelsman (7), who give from 0 to 2 cells per cmm. as the cell count of lumbar fluids in dogs.

The average cell count of lumbar fluid as found by us was 1.8 cells greater than the average for cistern fluid. This difference was 5.0 times as large as its probable error (even with the use of the incorrect formula which gives too large a value to the probable error of the difference), indicating that the difference was a real one rather than a chance one. Moreover, in those twenty dogs in whom both lumbar and cistern fluids were obtained, the lumbar cell count was greater than the cistern in seventeen instances.

A similar observation that in the human subject there are normally more cells per cubic millimeter in the lumbar than in the cistern fluid has been made by Schönfeld (8) and by Kafka (9).

The cells in the normal cerebrospinal fluid, as in the human, are practically all lymphocytes.

SUMMARY

1. Normal cerebrospinal fluid in dogs is clear, colorless, limpid and devoid of bactericidal activity. The amount of fluid obtained ranged in

forty-five cistern punctures from 1 to 8 cc., with an average of 3.4 ± 0.17 cc.; in twenty-seven lumbar punctures it ranged from 0.5 to 5 cc., with an average of 2.2 ± 0.22 cc. The 1.2 cc. difference between the average amounts obtainable by cistern and lumbar puncture is statistically significant.

2. The cerebrospinal fluid pressure in dogs under morphine-ether anesthesia ranged from 3 to 23 cc., with an average of 14.3 ± 0.53 cm. of water, in thirty-seven cistern punctures; in twenty-two lumbar punctures it ranged from 5 to 18 cm. with an average of 12.3 ± 0.53 cm. of water. This 2.0 cm. of water difference between the averages for cistern and lumbar pressure is statistically not significant.

3. In eighteen determinations in eleven dogs, Ayala's "Quotient Rachidien" varied from 0.33 to 1.37 with an average of 0.70 ± 0.04.

4. The cells in normal fluid are all lymphocytes. The number of cells varied between 0 and 10 with an average of 2.2 ± 0.20 in fifty-eight cistern punctures; in thirty-nine lumbar punctures it ranged from 0 to 10 with an average of 4.0 ± 0.30. The 1.8 difference between the means of cistern and lumbar fluid cell counts is statistically significant.

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OXYGEN USAGE OF UTERINE MUSCLE OF THE SOW[1]

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Various observations have shown that the isolated plain muscle of warm blooded animals has a low oxygen metabolism. A review of this work is given by Evans (1923), (1926) who used, in his own investigations, a modification of Winterstein's microspirometer. He found that the muscle of the uterus and of the intestine of the guinea pig and of the rabbit used from 0.30 to 0.37 cc. of oxygen per gram per hour, while that from the duodenum of the cat was as high as 0.45 cc. He says that this may be due to its more rapid rhythmic contraction as compared with the more slowly contracting uterus. Sometimes the muscle was separated from the mucosa, and in other cases an estimated deduction was made for the oxygen used by the mucous membrane.

Now that there are other delicate methods available for studying tissue metabolism, it seemed worthwhile to consider the oxygen consumption of uterine muscle, by a series of experiments which might demonstrate its relation to the phases of the activity of this organ.

It has been shown repeatedly that the character of the contraction of the muscle of the uterus in vitro bears a relation to the time in the oestrous cycle of the animal. Keye's (1923) findings for the pig's uterus are, in general, typical. The contractions are fairly rapid, regular and of large amplitude during oestrus and until the ova have passed into the uterus; they become rapid, irregular and low, and then slow but still of small amplitude during the following periods of the cycle. Reynolds (1930) has also demonstrated periodicity in the movements of the uterus in the living unanesthetized rabbit.

The muscle of the sow's uterus was used in this investigation. After the report was completed, there appeared two papers on the oxygen metabolism of the uterus in relation to the cycle. Khayyal and Scott (1931) used the isolated uteri of the mouse and rat, employing a modified Barcroft manometer. They found that the oxygen consumption was

approximately the same during the greater part of dioestrus and oestrus. An enormous increase occurred between about 40 and 24 hours before the onset of oestrus.   They found a sharp rise an hour after the subcutaneous injection of oestrin, which induced artificial oestrus in ovariectomized mice and a similar rise on the addition of oestrin to the Ringer-Locke solution which bathed the uterus.   David (1931) also used the uterus of the mouse. He reports "no definite increase in oxygen use in natural oestrus or when oestrus was induced in the castrated animal" but he found a definite increase when it was induced by injection of oestrin into the immature mouse.

MATERIAL AND METHOD. The muscle of the sow's uterus was used because abundant material in all stages was available and because Corner's work (1921) gives a method for accurately dating specimens.   It has certain advantages over that of the mouse and rat because of the 21 day cycle and the long period of oestrus.   There is, however, the disadvantage that the wall is thick and must be divided.   It has been shown (King, 1927) that the separated longitudinal layer gives the same type of contraction as the entire wall but with accentuation of amplitude and of tone.   This layer was therefore used.

Determinations were made by Fenn's modification of the Thunberg microspirometer (1927a) but the set-up varied from his in that no stimulating electrodes were needed and the temperature of the automatically regulated bath was 37.5°.   The capacity of the capillaries of the different respirometers varied in volume from 0.0028 to 0.0026 cc. per cm. and the capacity of the bottles was close to 26 cc.   Oxygen was passed through the respirometer just before placing the apparatus in the water-bath.

The uteri were received from the slaughter-house while still warm and were kept in the refrigerator except when samples were being taken. Whether this period was a few minutes or several hours, the results were similar.   Occasionally determinations were made after 24 hours and these checked well with those of the previous day.   The part of the uterine horn 20 to 40 cm. from the tubal end was selected and two longitudinal incisions were made 2 to 3 mm. apart.   The longitudinal layer of muscle was dissected from the circular and was caught by fine pointed forceps and stripped down for about 3 cm.   Sometimes, if the longitudinal layer itself could be split without too much injury, a still thinner preparation was made. Since the stimulus of cutting causes immediate contraction, measurements made after 20 minutes or more in Locke's are more reliable.   Within an hour after placing the apparatus in the bath, the muscle has shortened appreciably and measurements made at the end of an experiment show that it is about half as long and about one-third broader, while the thickness of the entire longitudinal layer, calculated from the area and weight, varied from 0.8 to 1.2 mm.   If there were irregularities in the shape of the

piece of muscle, which became apparent only when it was in a highly con-
tracted state, accurate measurements were very difficult.

In the early experiments, grouped as series A, the muscle was weighed
on a torsion or a quantitative balance and attached as quickly as possible
to a platinum hook on the stopper of the respirometer bottle.   Later, it
was also weighed at the end of the observation period but, for uniformity,
calculations were based on the initial weights.   After the publication of
the observations of Duliere and Horton (1929) on *Reversible Inexcitability
in Muscle*, another group of experiments, series B, was carried out.   In
this the muscle was prepared as above but was placed immediately in
aerated Locke's solution kept at room temperature.   After from fifteen
minutes to an hour it was removed, quickly weighed on a quantitative
balance, occasionally measured and then suspended in the respirometer
bottle.   It was again weighed at the end of the observation period and
also measured.   This second wet weight was used in these calculations.
The original weights of from 50 to 80 mgm. were reduced 20 to 40 per
cent.   This per cent of decrease is in general agreement with the loss
reported by Fenn (1927b) for nerves and attributed by him to the draining
away of blood and lymph.   The greatest loss was, as would be expected,
in muscles washed in Locke's solution.   A minimum observation period
was allowed after the index drop of kerosene began moving with regularity,
since cutting and handling increases the metabolism of this as well as of
other tissues, as stated by Fenn (1927b) and Adolph (1929).

The ovaries and uteri were examined when received and ova were looked
for in the tubes and uteri, if the condition of the corpora lutea indicated
their presence.   Blocks of ovary and uterus were fixed in Bouin's solution
and all specimens were dated by the histological method.

RESULTS AND DISCUSSION.   The determinations included in this report
were based on thirty-four experiments in series A and twenty-four in
series B.   The results of the first series were very disappointing because
of the irregularities within a period which brought the averages of the
oxygen consumed during the very active and the relatively quiescent
periods close together.   The second group of experiments, series B, gave
much more significant results.   As has been said, the specimens used in
these experiments were first washed in Locke's solution.   The 24 deter-
minations were distributed over the oestrous cycle in the following
manner: 6 within the first 3 days of the cycle, when the eggs were in the
tubes; 5 taken from 4 to 7 days after ovulation, while the ova were degen-
erating in the uterus; 7 in the 8 to 18 day period, during which time the
corpora lutea reach the height of their activity and then retrogress; 6
covered the last 3 days of the cycle, the time when the Graafian follicles
are rapidly maturing, preparatory to a new ovulation.   Table 1 gives the

condition of the specimens and the oxygen consumed, according to the day of the cycle. Table 2 gives the averages for each phase. Some of the determinations were made with two respirometers. These usually checked well though there were sometimes differences which could not be accounted for. Adolph (1929) mentions the fact that different pieces of skin from the same frog might show surprising variations.

The oxygen metabolism correlates, however, with the activity of the muscle as shown by the records of its contraction in vitro. It is the greatest during the last 3 days of the cycle, falls somewhat during the next 3 days and reaches the lowest averages from 4 to 18 days after ovulation. Separated longitudinal strips taken from the last period show a remarkable

Table showing the data for individual expe⟨…⟩on wh⟨…⟩

	36	20	30	23	26	31	35	32	33	34	19	25
Day of cycle.............	1	2	2	2	3	3	4	6	6–7	6–7	7	10
Ova recovered from....	Tubes	Tubes	Tubes	Tubes	Tubes	Tubes	Uterus	Uterus	Uterus	Uterus	Not foun⟨…⟩	
Condition of and size of corpora lutea	Very early	5 mm.	5–6 mm.	5–6 mm.	6 mm.	6 mm.	Hemor-rhagic, 6 mm.	Hemor-rhagic, 8 mm.	Almost solid, 8 mm.	Not solid, 8 mm.	7–8 m⟨…⟩ mm.	
Size of follicles..........	2 unrup-tured, 9 mm.											
Cubic centimeters oxy-gen consumed per gram of muscle per hour.................	0.6105	0.5430	0.5131	0.5119	0.5306	0.4258	0.5031	0.3950	0.4318	0.4872	0.33⟨…⟩	4690

degree of tone and yet the low metabolism is consistent with Evans' (1923) findings that the muscle of the intestine and of the uterus does not use more oxygen when in tonus.

The question may be raised as to whether the difference in oxygen usage at differenct periods may not be due to the variations in the thickness of the muscle strips. The thickness of tissue which could be adequately supplied by oxygen at the determined rate of consumption was calculated according to Warburg's formula (1930). In table 3 these thicknesses are compared with those determined by the area and weight of the muscle. The thickness of the 4 to 7 day specimens can possibly be accounted for by irregularities in the shape of the piece, which made accurate measurements difficult. However, assuming that the thickness was 1.5 mm. the part of the muscle actually respiring would use oxygen at the rate of 0.498 cc. per hour, a rate appreciably below that of the oestrous period.

The change in the length of the muscle strip at the end of an observation

period was the result of the normal contraction and was not due to rigor mortis. The oxygen used would increase with the onset of rigor and would result in gross error. After weighing the muscle at the close of an experiment, its elasticity was almost always demonstrated by stretching. In a few cases the extent of stretching was measured, the muscle replaced in Locke's and the time before contraction occurred noted. It is well known that uterine muscle in oxygenated Locke's solution, kept at body temperature, will continue to contract for many hours but the condition is not the same with the specimen suspended in an atmosphere of moist oxygen. It would be instructive to have a simultaneous graphic record of the same muscle on which oxygen usage was being studied.

dual exper... on which the averages for table 2 are based

...s of experiments														
4	19...	24	27	37	25	29	41	39	38	40	21	43	44
-7 rus	7 Not foun	10	12	13–14	15	16	17	16–17	19	20	20–21	21	21	21
id, am.	7-8 m... mm.	10 mm.	10 mm.	Retrogressing, 8-9 mm.	Retrogressing, 8 mm.	Retrogressing, 6–7 mm.	Retrogressing, 5 mm.	5 mm. 7 mm.	None 9 mm.	5 mm. 8 mm.	None 7 mm.	None 8 mm.	None 9 mm.
72	0.33... 469	0.4040	0.4400	0.322	0.370	0.412	0.442	0.5230	0.6943	0.8050	0.770	0.6362	0.7988

There is an interesting parallelism between the working power of the uterine muscle and its oxygen metabolism. It was found (King, 1927) that the mechanical activity was lowest from the 4th to the 17th day after ovulation and that it began to rise on the 17th to the 18th day and reached a maximum on the 1st and 2nd days of the cycle. This study places the greatest oxygen usage on the last 3 days but the low level is in agreement with that of greatly diminished working power. It should be noted, however, that as delicate a method was not possible for studying the working power as for that of determining oxygen metabolism.

It is significant that the retrogression of the corpora lutea, which begins about the 15th day, is not the signal for a rise in metabolism. It was as low when these bodies were only scar tissue as when they were at the height of their activity. The increase comes when the follicles reach 7 to 8 mm. in diameter, indicating that they are the controlling factor. Additional support is given to this by the results from one experiment, no. 36. This

specimen was dated on the first day of the cycle. The ova had just been discharged, as evidenced by the collapsed walls of the follicles and the almost complete absence of lutein tissue. Two large follicles had not ruptured, although in microscopic section they appeared normal. The rate of 0.6105 cc. of oxygen per gram per hour places it in the preceding group, when the uterus was still under the influence of estrin. Indeed this influence must not be lost entirely until the third or fourth day after ovulation, since on the third day it is still above the interval level. These results are significant in the light of Reynold's recent report (1931). By injecting theelin into castrated rabbits, quiescent uteri were aroused to activity.

TABLE 2

Table showing the average amount of oxygen consumed by the uterine muscle of the pig according to the phase of the cycle

NUMBER OF SPECIMENS	DAYS AFTER OVULATION	AVERAGE OXYGEN USAGE PER GM. PER HR. OF MUSCLE	DEVIATION FROM MEAN OF HIGHEST	DEVIATION FROM MEAN OF LOWEST
			per cent	per cent
6	1–3	0.5292	14.3	19.5
5	4–7	0.4372	15.0	15.5
7	8–18	0.4050	10.4	20.5
6	19–21	0.7045	14.2	25.6

TABLE 3

Period in days	1–3	4–7	8–18	19–21
Measured thickness in millimeters	0.8	1.5	1.3	1.06
Thickness which could be adequately supplied with oxygen at the determined rate of consumption	1.1	1.24	1.28	0.97

He considers that the follicular hormone is responsible for the oestrous type of uterine contraction.

The changes in the oxygen metabolism are slight, when compared with the findings of Khayyal and Scott (1931) for the mouse and rat but they are more definite than the results of David (1931) on the normal adult mouse.

CONCLUSIONS

The oxygen metabolism of the uterine muscle of the sow was determined by Fenn's modified microspirometer. It varies with the phase of the oestrous cycle, being greatest during the three days before ovulation and lowest between the fourth and eighteenth days.

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STUDIES OF GALL-BLADDER FUNCTION[1]

IV. The Absorption of Chloride from the Bile-Free Gall Bladder

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Laurentius Heister (1728) stated that gall-bladder bile was more viscid than liver bile and MacLurg (1772) in the first monograph published on the bile made similar observations, stating that "the gall-bladder bile was more viscid and bitter than liver bile." These observations found confirmation later in studies of the total solids of liver and gall-bladder bile by Maly (1881) and Brand (1902). However, it was not until Rous and McMaster (1921) demonstrated changes in the pigment content of liver bile, once this entered the gall bladder, that we had any definite quantitative data on the ability of the gall bladder to concentrate liver bile.

Numerous studies on the concentrating function of the gall bladder have been made since Rous and McMaster and their associates (1921, 1924) published their papers, but on the whole, they have added little to the subject. No attempt has been made by one group to study a number of the individual constituents of the bile in a bile free gall bladder or to carry this study still further to the fate of these substances in liver bile once it enters the gall bladder. References to studies of other investigations on the fate of certain bile constituents in the gall bladder will be given when these constituents are separately reported on.

Suffice it to say that most previous studies are open to criticism from the standpoint of approach and method. Puncture of the gall-bladder wall results in increased absorption (Winkenwerder, 1930). Ligation of the cystic duct injures not only the lymphatics, but frequently interferes with the vascular supply of the gall bladder. In other studies the presence of accessory ducts entering the gall bladder or cystic duct has not been excluded so that a known amount of bile placed in the gall bladder may have had added to it hepatic bile and the true absorptive function may not have been fully demonstrated. Since Rous and McMaster do not mention the exclusion of accessory ducts in their studies it is possible that

the maximum concentrating function of the gall bladder has not been completely demonstrated.

It seemed logical that a study of the absorptive function of the gall-bladder wall in regard to bile might be begun by a study of the fate of various concentrations of sodium chloride, calcium lactate, cholesterol, sodium glycocholate and sodium taurocholate when placed separately or combined in the gall bladder of the dog, the gall-bladder lymphatics and blood vessels remaining intact, but the ductal connections with the liver being severed.

In this paper we are reporting the results of experiments in which varying concentrations of sodium chloride or sodium chloride and sodium bicarbonate were used. In these studies we have followed volume changes as well as changes in concentration and composition.

METHOD. Dogs weighing from 12 to 26 kilograms were anesthetized with ether and operated on under careful aseptic technic. The abdomen was opened through a paramedian rectus incision and the extra-hepatic biliary passages carefully exposed. The anatomic relations of the cystic blood vessels and visible accessory ducts were ascertained. A ligature was placed on the duct formed by the union of the central and cystic ducts just above the points of entrance of the right and left

Method of Cannulating Gall Bladder

Fig. 1

lobe ducts (fig. 1). The central lobe duct was then ligated at a point which would not interfere with any blood vessels going to or coming from the gall bladder.

The main duct was slit just above the ligature and a soft rubber catheter, size 8 to 12 French, was inserted through this opening and passed from this point through the cystic duct into the gall bladder. It was secured by a second ligature. Every accessory duct which could be visualized was ligated if this was possible without injury to the major lymphatics or blood supply. If this was impossible the animals were discarded.

The gall bladder was irrigated through the catheter with physiologic saline solution until the fluid returned clear. A known amount of solution was then introduced into the gall bladder, the exact amount varying in the different dogs according to the size of the gall bladder as determined when the gall bladder was first aspirated. Before closing the abdomen we assured ourselves that the amount introduced could be recovered.

Complete emptying was determined by the introduction of air and its subsequent aspiration free from fluid.   The major portion of the catheter was wrapped in omentum and the terminal portion brought out through a stab wound to the right of the incision or through the lower end of the incision.   The wound was closed by tier suture.

A known amount of physiologic sodium chloride was left in the gall bladder for from one to two hours.   If an accessory duct had been overlooked the solution became bile stained within that time.   When this occurred the animals were discarded.

When the bile removed from the gall bladder was quite inspissated it was necessary to wash out again after one to two hours in order to remove pigment which may cling tenaciously to the gall-bladder wall.   From our experience we do not believe it is possible to determine complete cleansing by observation through the gall-bladder wall, although this has been suggested (Rous and McMaster, 1921, p. 52).   The catheter was kept closed by a Hoffman clamp and sterile dressings were kept over the wound constantly.   A muslin binder covered the abdomen and thorax in such a way that the dog could not get at the wound.

Several conditions are essential for a successful experiment: 1, the maintenance of asepsis; 2, the avoidance of damage to the vascular supply or to the major gall-bladder lymphatics; 3, the prevention of injury to the right and left lobe ducts so that hyperbilirubinemia does not occur; 4, the ligation of all accessory ducts which may pour hepatic bile into the gall bladder after the critical ligatures have been placed.

With this type of preparation we were able to make volume, concentration, and composition studies of the solutions used.   The period of study of the animals used in these experiments varied considerably.   In some experiments the solution was not aspirated for a number of hours, while in others samples were removed every hour or two.   In still others we temporarily removed the contents of the gall bladder merely for estimation of volume changes and immediately reinjected the solution.   The animals were finally killed and in a number of instances the gall bladder was removed for histologic study.   The following methods were used in the analyses made in these experiments.   Chlorides were estimated by the Wilson and Ball (1928) modification of the Van Slyke method.

Total base was measured by the method of Stadie and Ross (1925).

Total CO_2 was measured by the method of Van Slyke and Neill (1924) using a Van Slyke constant volume apparatus.

Potassium was measured by the titrimetric method of Shohl and Bennett (1928).

Phosphates were estimated by an adaptation of the method of Fiske and Subbarow (1925).

Calcium was determined by the method of Kramer and Tisdall (1921).

Carbonate was measured by titration with N/50 HCl with phenolph-thalein as indicator and bicarbonate determined from total CO_2 by differ-ence. Titration for carbonate was not performed in all experiments. Carbonate was not in excess of a few m.Eq. per liter except when total CO_2 was above 50 mM per liter. When, therefore, carbonate had not been determined, total CO_2 was assumed to be bicarbonate in estimating total anion.

RESULTS. *The absorption of water.* When a solution of sodium chloride or a mixture of sodium chloride and sodium bicarbonate was introduced into the gall bladder there resulted a decrease in the fluid content except when hypertonic solutions were used. When the latter were introduced there was an initial period during which fluid entered the gall bladder but this was followed by removal of water after the concentration of the solu-tion, either by dilution or absorption of salt, or both, approached 170 m. Eq. per liter.

When water begins to leave the gall bladder, it continues to do so as long as the gall-bladder wall remains normal. This function of the gall bladder is so constant that one must consider it as of great importance when at-tempting to determine normality of the membrane.

The gall bladder removed fluid rapidly at first. This period varied from 21 to 48 hours. After this there occurred a period of varying length during which the rate of absorption of fluid was slower. This was followed by a period which came on in one instance as late as 141 hours after intubation of the gall bladder, when fluid began to pour into its lumen. The amount of fluid removed in the first, or active period averaged between 3 and 4 cc. per hour when estimated for a complete active period, except when hypo-tonic solutions were used, when the average rate of outflow during an active period was 5.4 cc. There was, however, considerable variation in different experiments. The highest rate of removal when all bile was excluded was 8 cc. per hour and the highest rate of inflow was 3.5 cc. per hour.

Although the absolute amount of fluid absorbed varied considerably in comparable periods in the individual dogs, the per cent removed bore a very much closer relationship. Consecutive experiments in which the amount of fluid introduced may have varied show a definite relationship when one considers per cent removal per hour.

Changes in composition of the fluid. In every experiment in which a normal membrane was being used, as evidenced by the removal of fluid, chloride was absorbed from the gall bladder. The rate of its absorption varied according to the concentration of the solution used. When isotonic solutions were introduced chloride and water left at about the same rate, so as to maintain a nearly constant concentration. When hypotonic solutions were used chloride tended to leave more slowly than water so

that the concentration of chloride usually increased. When hypertonic solutions were used we occasionally found an increase of the total chloride during the first period when fluids were pouring into the gall bladder. However, in the majority of instances the total chloride had been reduced even during the first hour after the introduction of a hypertonic solution and the rate of chloride absorption exceeded that of water until isotonicity was reached.

TABLE 1

Dog 525.   Weight, 8.2 kgm.   January 19, 1931.   Sodium chloride

TIME	VOLUME IN	VOLUME OUT	Cl CONCENTRATION IN	Cl CONCENTRATION OUT	Cl IN	Cl OUT	Cl CHANGE	TOTAL CO₂ IN		TOTAL CO₂ OUT		MEASURED ANION IN	MEASURED ANION OUT
hours	cc.	cc.	m.Eq. per liter	m.Eq. per liter	m. Eq.	m.Eq.	m.Eq.	mM per liter	mM	mM per liter	mM	m.Eq. per liter	m.Eq. per liter
1	15.0	18.5	252	234	3.78	4.32	+0.54	0.3	0.005	5.2	0.96	252.3	239.2
1	16.0	16.5	234	206	3.75	3.41	−0.34	5.2	0.083				239.2
1	15.5	13.5	206	172	3.20	2.33	−0.87			9.3	0.126		181.3
1½	11.5	9.0	172	142	1.98	1.28	−0.70	9.3	0.107	14.4	0.130	181.3	156.4

TABLE 2

Dog 474.   Weight, 18.8 kgm.   January 13, 1931.   Sodium chloride

TIME	VOLUME IN	VOLUME OUT	Cl CONCENTRATION IN	Cl CONCENTRATION OUT	Cl IN	Cl OUT	Cl CHANGE	TOTAL CO₂ IN	TOTAL CO₂ OUT	MEASURED ANION IN	MEASURED ANION OUT	TOTAL BASE OUT
hours	cc.	cc.	m.Eq. per liter	m.Eq. per liter	m.Eq.	m.Eq.	m.Eq.	mM per liter	mM per liter	m.Eq. per liter	m.Eq. per liter	m.Eq. per liter
1	30.0	27.5	167	155	5.0	4.25	−0.75	0.1	5.8	167.1	160.8	
1	25.0	19.0	155	144	3.88	2.73	−1.15	5.8	10.8	160.8	154.8	
1	16.8	13.5	144	130	2.42	1.76	−0.66	10.8	19.9	154.8	149.9	154*
1	30.0	26.0	165	151	4.95	3.93	−1.02	0.3	6.1	165.3	157.1	
1	23.0	20.0	151	134	3.47	2.68	−0.79	6.1	17.6	157.1	151.6	153†

* K 1 m.Eq. per liter.

† Ca 1.5 m.Eq. per liter.

In dog 525 (table 1) the solution introduced into the gall bladder contained 252 m.Eq. of chloride per liter. During the first two periods fluid poured into the gall bladder and in the first hour the total chloride in the gall bladder increased. In each subsequent period chloride left the gall bladder more rapidly than water resulting in a decrease in the chloride concentration. Bicarbonate entered the solution slowly, so that the anion concentration fell constantly during the experiment, approaching

the total anion concentration of serum four and one-half hours after the introduction of the fluid.

In table 2 the data on dog 474 are given. In this experiment the solution of sodium chloride used was approximately 167 m.Eq. per liter. As the chloride concentration decreased bicarbonate supplanted the chloride in part; but the concentration of the anions introduced was always slightly in excess of those removed. In this experiment water was also leaving the gall bladder more slowly than was chloride, so that the concentration of chloride was constantly falling.

TABLE 3

Dog 607. February 17, 1931. Weight, 14.9 kgm. Sodium chloride

TIME	VOLUME IN	VOLUME OUT	Cl CONCENTRATION IN	Cl CONCENTRATION OUT	Cl IN	Cl OUT	Cl CHANGE	TOTAL CO_2 IN	TOTAL CO_2 OUT	MEASURED ANION IN	MEASURED ANION OUT
hours	cc.	cc.	m.Eq. per liter	m.Eq. per liter	m.Eq.	m.Eq.	m.Eq.	mM per liter	mM per liter	m.Eq. per liter	m.Eq. per liter
1	40.0	32.0	129.7	127.7	5.20	4.09	−1.11	0.6	12.8	130.3	140.5
4	28.0	3.5	127.7	107.3	3.57	0.38	−3.19	12.8	23.5	140.5	130.8

TABLE 4

Dog 96. Weight, 13.2 kgm. September 10, 1930. Sodium chloride

TIME	VOLUME IN	VOLUME OUT	VOLUME CHANGE	Cl CONCENTRATION IN	Cl CONCENTRATION OUT	Cl IN	Cl OUT	Cl CHANGE	BLOOD SERUM CHLORIDE	
									At start	At end
hours	cc.	cc.	cc.	m.Eq. per liter	m.Eq. per liter	m.Eq.	m.Eq.	m.Eq.	m.Eq. per liter	m. Eq. per liter
1½	20.0	10.5	−9.5	68.4	106.0	1.37	1.11	−0.26	114.5	114.5
1½	7.5	3.7	−3.8	106.0	111.1	0.80	0.41	−0.39	114.5	111.1

Total base was estimated at two points during the experiment. In each instance it was higher than the estimated total anions. Potassium and calcium estimations were each made in one instance. It can be inferred that the total base was made up nearly entirely of sodium.

In table 3 are given the data for a five hour period of dog 607. The solution used was approximately 130 m.Eq. per liter. For the first hour water and chloride left the gall bladder at nearly the same rate while in the succeeding four hours chloride left more rapidly than water. This resulted in a reduction of the chloride concentration by about 23 m.Eq. per liter and a loss of 4.30 m.Eq. of chloride from the gall bladder. The bicarbonate rose during the five hour period from 0.6 m.Eq. per liter to 20.5 m.Eq. per liter, since 3 mM per liter of a total CO_2 of 23.5 mM per liter was

carbonate. At the end of the five hour period the chloride lost was replaced by carbonate and bicarbonate so that the measured anion concentration was the same as at the beginning of the experiment.

When a hypotonic solution of sodium chloride is placed in the gall bladder the concentration tends to approach the serum level for chlorides. In dog 96 (table 4) the data are given for a three hour period when such a solution was used. Throughout the period water was absorbed more rapidly than chloride and the chloride concentration increased, until at the end of the period the chloride concentration of the solution and serum were identical. The time necessary for this to occur depends on the hypotonicity of the solution and the amount of fluid in the gall bladder.

In table 5 (dog 604) are given the data when a mixture of sodium chloride and sodium bicarbonate with a total estimated anion concentration of 136 m.Eq. per liter was introduced into the gall bladder. The chloride and bicarbonate concentration both decreased slightly but the rate of

TABLE 5

Dog 604.   February 3, 1931.   Weight, 17.0 kgm.   Sodium chloride-sodium bicarbonate

TIME	VOL-UME IN	VOL-UME OUT	Cl CON-CEN-TRA-TION IN	Cl CON-CEN-TRA-TION OUT	Cl IN	Cl OUT	Cl CHANGE	TOTAL CO$_2$ IN	TOTAL CO$_2$ OUT	MEAS-URED ANION IN	MEAS-URED ANION OUT	TOTAL BASE OUT
hours	*cc.*	*cc.*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq.*	*m.Eq.*	*m.Eq.*	*mM per liter*	*mM per liter*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq. per liter*
2	30.0	14.0	88.7	87.1	2.67	1.21	−1.46	47.4	43.7	136.1	130.8	
1	10.0	6.0	87.1	85.5	0.87	0.51	−0.36	43.7	43.1	130.8	128.6	139

change over a three hour period was indeed small. At this concentration of chloride and bicarbonate the anions are absorbed so as to maintain approximately the same concentration. When mixtures of sodium chloride and sodium bicarbonate were used in the concentrations used in this experiment the chloride concentration did not increase during the short period of study.

The damaged gall bladder. When the gall bladder of the dog, prepared as already described, is damaged as the result of infection or from the continued introduction of sodium chloride, especially in hypertonic concentrations, the results are entirely different from those obtained from the normal gall bladder. The damaged gall bladder absorbs fluid slowly for a time and then pours fluid into its lumen. The changes in chloride concentration after the introduction of sodium chloride are slower but the concentration of bicarbonate and carbonate goes to very much higher levels. The anion concentration after a period of hours has varied from about 136 to 160 m.Eq. per liter.

In table 6 (dog 565) this change is well illustrated. The chloride was introduced in a concentration of approximately 160 m.Eq. per liter. There occurred constantly a loss of chloride and an increase in the bicarbonate and carbonate in sufficient concentration to maintain an anion concentration of these three ions between 136 and 148 m.Eq. per liter. In three instances total base estimations were made on the recovered solutions and it can be seen that chloride and total CO_2 account for most of the anion present in the solution.

In table 6 (dog 548) the gall bladder was left empty after fluid began to pour into it. The concentration of chloride in the solution pouring in

<div align="center">

TABLE 6

The damaged gall bladder

When the gall bladder is damaged fluid pours into it

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TIME	VOL-UME IN	VOL-UME OUT	Cl CON-CEN-TRA-TION IN	Cl CON-CEN-TRA-TION OUT	Cl IN	Cl OUT	Cl CHANGE	TOTAL CO_2 IN	TOTAL CO_2 OUT	MEAS-URED ANION IN	MEAS-URED ANION OUT	TOTAL BASE IN	TOTAL BASE OUT

<div align="center">

Sodium chloride

Dog 565

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hours	*cc.*	*cc.*	*m.Eq. per liter*	*m.Eq. per liter*	*m. Eq.*	*m.Eq.*	*m.Eq.*	*mM per liter*	*mM per liter*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq. per liter*
16½	20.0	7.5	160.0	102.8	3.19	0.77	−2.42	0.0	32.9	160.0	135.7	160	
4	10.0	10.0	156.3	117.7	1.55	1.18	−0.37	0.0	25.6	156.3	143.3		
4	10.0	12.0	158.7	112.2	1.59	1.52	−0.07	0.0	35.6	158.7	147.8		150
16	10.0	13.0	160.0	66.7	1.59	0.87	−0.72	0.0	79.5	160.0	146.2		148
6½	15.0	15.5	160.3	82.4	2.43	1.28	−1.15	0.0	61.6	160.3	144.0		146

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Dog 548

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TIME	VOL-UME IN	VOL-UME OUT	Cl CON-CEN-TRA-TION IN	Cl CON-CEN-TRA-TION OUT	Cl IN	Cl OUT	Cl CHANGE	TOTAL CO_2 IN	TOTAL CO_2 OUT	MEAS-URED ANION IN	MEAS-URED ANION OUT	TOTAL BASE IN	TOTAL BASE OUT
8	0.0	5.0	0.0	95.0				0.0	58.8		153.8		
15½	0.0	7.0	0.0	98.3				0.0	56.5		154.8		160
24	0.0	19.0	0.0	100.4				0.0	63.3		163.7		
26	0.0	23.0	0.0	98.9				0.0	64.9		163.8		

was between 95 and 100 m.Eq. per liter, while the total CO_2 concentration varied between 59 and 65 m.Eq. per liter.

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SUMMARY

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The experiments reported here have all been done in the gall bladder which was free of bile. In this type of gall bladder if the lymphatics and blood supply have not been injured and if the gall bladder has not been damaged by trauma or infection, water is rapidly absorbed. The rate of absorption varies in individual animals.

Regardless of the state of the gall bladder, whether water is being rapidly or slowly absorbed, or whether fluids are pouring into a gall bladder which is being emptied at intervals, the total osmolar concentration of the fluid found in the gall bladder after a period of hours approaches that of the serum.   The rapidity with which this occurs depends largely on the concentration initially introduced.

When hypertonic solutions are used water pours into the gall bladder. With this may come some chloride but as a rule total chloride has been found to be reduced while water was still pouring in, indicating that chloride can leave while water flows into the gall bladder.

Whether the solution initially introduced is sodium chloride or a mixture of sodium chloride and sodium bicarbonate, the anion concentration of the fluid becomes composed mainly of chloride and bicarbonate with traces of carbonate and phosphate.

The ratio of bicarbonate to chloride attained in these experiments varies greatly and appears to become stabilized at widely varying values from 0.1 to 1.7, further changes occurring very slowly.   The highest bicarbonate concentrations were found in those fluids which were being removed very slowly or which were being added to by a gall-bladder wall which had reversed the normal process of water transfer.   In such a gall bladder, concentrations of total CO_2 have been found as high as 88.4 m.M per liter. The carbonate increased very slowly, the highest ever found in the gall-bladder contents being 13.9 m.Eq. per liter.   Phosphate has never exceeded 0.5 m.Eq. per liter.

The base in the solutions remains nearly entirely sodium.   The highest concentration of potassium has been 4.3 m.Eq., while the highest for calcium was 2.5 m.Eq., out of a total of about 150 m.Eq. per liter.   The cation studies were not made in every experiment but sufficient studies were made to permit us to say that marked variation from these findings is unlikely.

The mechanism involved in the later phases of many of these experiments when fluids were pouring into the gall bladder is, we believe, associated with damage to the gall-bladder wall with a resultant perversion of function.   The histologic studies of the gall-bladder wall in these cases show droplet formation of the mucosal cells, infiltration of polymorphonuclear leukocytes into the submucosa and a thickening of the serosa.

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STUDIES OF GALL-BLADDER FUNCTION[1]

V. The Absorption of Calcium from the Bile-Free Gall Bladder

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That the calcium content of bile is altered by the gall bladder has long been evident from a comparison of the calcium content of liver and gall-bladder bile (Hoppe-Seyler, 1878; Drury, 1924).

Since calcium is a common constituent of gall stones, the action of the gall-bladder wall on solutions containing calcium is an important one. That the gall bladder absorbs water at a rapid rate is a well known fact (Rous and McMaster, 1921), but the absorption of calcium by the gall bladder has not previously been studied under adequately controlled conditions.

The studies in this report are a part of a general study of the absorption of various constituents of bile from the gall bladder and are concerned with changes brought about in calcium lactate solutions placed in the gall bladder, whose ductal connections with the remainder of the biliary system have been completely occluded, but whose lymphatic and blood supplies are intact. The method has been described and discussed previously (Ravdin, Johnston, Austin and Riegel, 1932).

METHOD. Our data were obtained from 22 dogs. Calcium lactate solutions varying in concentration from 3 m.Eq. per liter to 100 m.Eq. per liter were introduced into the gall bladder and allowed to remain for varying periods of time. The solutions introduced and recovered were analyzed for calcium (Clark and Collip, 1925) in all instances. In addition, analyses for chloride (Wilson and Ball, 1928), total CO_2 (Van Slyke and Neill, 1924) and lactate (Friedemann, Cotonio, and Shaffer, 1927) were done on the solutions from four animals. Total base (Stadie and Ross, 1925), potassium (Shohl and Bennett, 1928) and sodium (Barber and Kolthoff, 1928) determinations were done on occasional specimens. Carbonate was measured by titration with N/50 HCl with phenolphthalein as indicator and bicarbonate determined from total CO_2 by difference.

Titration for carbonate was performed in most of the experiments. Carbonate was not in excess of a few m.Eq. per liter. When carbonate had not been determined total CO_2 was assumed to be bicarbonate in estimating total anions.

RESULTS. *The absorption of calcium from the normal gall bladder.* In the first few days during which an animal was used for these experiments, the day solutions recovered from the gall bladder had, in nearly every instance, a higher concentration of calcium than those introduced. The degree of concentration varied between wide limits, and this was true whether the solutions introduced had a calcium concentration of 3 m.Eq. per liter or 100 m.Eq. per liter.

For the purpose of more clearly presenting our data, we have considered that the gall bladder was normal only so long as it could concentrate the calcium in the solution. Later on, even during day periods, the rate of calcium disappearance increased so as to exceed the rate of water absorption, thus decreasing the calcium concentration of the solutions. This was a reversal of the initial relationship between water and calcium absorption during day periods. At this time the gall bladder was considered abnormal. Whether this change in permeability to the calcium ion constitutes an abnormal condition is open to question, since a similar variation was found during the night period, even when the specimens removed during the following day period indicated that the gall bladder was concentrating the calcium in the solution. That calcium was removed from the solution even when the concentration increased is indicated by the fact that the recovered solution contained less total calcium than that introduced. No evidence has ever been obtained by us which would indicate that calcium is being secreted into the normal gall bladder.

A typical protocol is given in table 1. During the first $55\frac{1}{2}$ hours of this experiment the calcium concentration increased during the day periods except for the first $2\frac{1}{2}$ hours following the operation at which the gall bladder was intubated. During the night periods the absorption of calcium was usually more rapid than the absorption of water, causing a decrease in the concentration of calcium in the solution.

Absorption of the lactate ion from the gall bladder. The lactate ion was usually absorbed from the gall bladder at a slower rate than was calcium (table 1). Lactate concentrations usually rose to a higher level than did the calcium concentrations, and were not subject to the marked diurnal variation seen in the calcium concentrations. There were exceptions to this as will be noted in table 1. In two night experiments the lactate concentration fell below that of calcium. It was in these experiments that the lowest calcium concentrations were observed. In an occasional period the ratio of calcium to lactate remained nearly constant.

Variations in total ion relationships brought about by the gall bladder. The

TABLE 1

Dog 995. Calcium lactate in gall bladder

	TIME	VOLUME		CALCIUM		LACTATE		TOTAL CO₃		CHLORIDE		MEASURED ANION		TOTAL CATION		CALCIUM		LACTATE	
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
	hours	*cc*	*cc*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq.*	*m.Eq.*	*m.Eq.*	*m.Eq.*
D	2½	25.0	18.0	24.8	20.6	26.1	25.3		9.5	2.3	90.5	28.4	125.3	26		0.62	0.37	0.65	0.46
D	3	20.0	9.0	24.8	33.4	26.1	37.4			2.3	78.8	28.4	116.2		109	0.50	0.30	0.52	0.34
N	17	30.0	19.0	26.2	14.3	24.3	31.5	1.9	12.7	3.3	104.7	29.5	148.9		148	0.79	0.27	0.73	0.60
D	9	20.0	10.0	26.9	27.6	25.3	37.1	1.0	15.7	0.4	102.8	26.7	155.6		152	0.54	0.28	0.51	0.37
N	16	20.0	25.0	26.9	3.4	25.3	2.8	1.0	22.2	0.4	136.1	26.7	161.1		165	0.54	0.09	0.51	0.07
D	8	20.0	10.0	26.9	30.3	25.3	46.0	1.0	18.5	0.4	85.1	26.7	149.6		145	0.54	0.30	0.51	0.46
N	16	20.0	11.0	26.9	6.1	25.3	24.8	1.0	24.3	0.4	103.7	26.7	152.8			0.54	0.07	0.51	0.27
D	6½	20.0	7.0	26.9	16.8	25.3	47.9	1.0	13.6	0.4	79.0	26.7	140.5			0.54	0.12	0.51	0.34
N	18	20.0	14.0	26.9	2.8	25.3	19.4	1.0	35.1	0.4	108.3	26.7	162.8		166	0.54	0.04	0.51	0.27
D	8	20.0	8.0	25.8	13.7	25.0	48.8	0.0	16.0	2.1	82.3	27.1	147.1			0.52	0.11	0.50	0.39
N	16	20.0	13.0	25.8	4.9	25.0	27.3	0.0	45.3	2.1	92.6	27.1	165.2			0.52	0.06	0.50	0.35
D	5	20.0	7.0	26.3	22.4	27.9	56.4	0.0	15.1	1.7	68.8	29.6	140.3			0.53	0.16	0.56	0.39
N	19	20.0	16.0	26.3	4.6	27.9	3.3	0.0	55.7	1.7	116.6	29.6	175.6		178	0.53	0.07	0.56	0.05
D	7½	20.0	3.0	26.3	5.5	27.9		0.0		1.7		29.6				0.53	0.02	0.56	
N	16	20.0	12.0	26.3	5.5	27.9	38.1	0.0	38.7	1.7	97.8	29.6	174.6	35		0.53	0.07	0.56	0.46
D	8½	20.0	7.0	39.0	21.4	33.4	57.5	0.0	17.8	4.2	72.5	37.6	147.8			0.78	0.15	0.66	0.40
N	17	20.0	10.0	39.0	10.9	33.4	50.0	0.0	36.9	4.2	74.8	37.6	161.7			0.78	0.11	0.66	0.50
D–N	22½	20.0	10.0	39.0	7.3	33.4	25.9	0.0	46.8	4.2	100.3	37.6	173.0		177	0.78	0.07	0.66	0.26
D	8	20.0	10.0	39.0	16.4	33.4	53.7	0.0	20.3	4.2	86.3	37.6	160.3			0.78	0.16	0.66	0.54
N	16½	20.0	6.0	39.0	9.7	33.4	38.8	0.0		4.2	84.6	37.6				0.78	0.06	0.66	0.23

D—Day period. N—Night period.

solution of calcium lactate introduced into the gall bladder in these ex-
periments was in all instances lower in total ion concentration than blood
serum.   The concentration of calcium however ranged from below blood
serum level to twenty times this level.   When solutions of calcium lactate
were placed in the gall bladder, total osmolar concentration tended to
approach that of serum (table 1).   The deficiency in base was corrected
almost wholly by sodium, the potassium at the time of removal of the
solution accounting for not more than 5 m. Eq. per liter of base.   This was
true whether the calcium concentration of the solution increased or
decreased.

Besides lactate, the anions consisted almost entirely of chloride and
bicarbonate.   The carbonate increased very slowly, the highest concen-
tration found in any experiment being approximately 4 m.Eq. per liter.
Traces of phosphate too low for quantitative determinations were occa-
sionally found.

There is apparently no correlation to be found between the concentra-
tions of lactate, chloride and bicarbonate in the solutions removed from
the gall bladder.   The sum of these ions as estimated approximated the
measured total base.

Of the anions, chloride tended to become stationary at serum level.
Bicarbonate values were more variable and tended to increase with the
length of time that the experiment continued.   The findings as regards
chloride and bicarbonate were similar to those reported by us in the paper
on sodium chloride absorption (1932).

Absorption of water from the normal gall bladder.   In 50 experiments on
22 dogs whose gall bladders we considered normal, the average hourly
absorption of water was 3 cc.   The average hourly absorption of water
during a night period was 1.2 cc., during a day period 3.7 cc.   There was
observed a decrease in the rate of water absorption after the gall bladder
had been used for experiments for several days, but the period of rapid
absorption persisted for a longer period of time than in the sodium chloride
experiments.   In dog 995 (table 1) water was still being absorbed 13 days
after the beginning of the experiment.

In the sodium chloride experiments the gall bladder was considered
normal until water was no longer absorbed.   In these calcium experiments
this fact cannot be taken as sole evidence of normality since water was
often being absorbed long after the gall bladder ceased to concentrate
calcium, even during a day period.   The general tendency was for the
gall bladder to absorb smaller and smaller amounts of water until finally
fluid entered the gall bladder.

There was a marked variation in the rate of water absorption during
the day and night periods.   This interesting fact had no relation to feeding
since the animals were usually fed about five o'clock in the afternoon,

which was considered the end of the day period.   When, however, animals
were kept awake during the night and volume changes estimated at fre-
quent intervals, the rate of absorption of water was similar to that ob-
served during the day period.   From this we have been led to conclude
that activity rather than feeding played the more important rôle in the
rate of water absorption.   Similar observations were obtained with solu-
tions of sodium chloride.

The variation in the rate of water loss influences the concentration of
calcium much less than it does bicarbonate or chloride.   The chief cause
of the greater decrease in the concentration of calcium during the night
periods is the increased absorption of calcium (table 1).

The damaged gall bladder.   In these experiments we have considered
the gall-bladder wall as abnormal when the calcium concentrations fell
during both the day and night periods.   Further damage was assumed
when reversal of function as regards water transfer had taken place.

When damage of the wall occurred we observed a rise of the total anion
concentration of the fluid in the gall bladder, the rise being largely due to
an increase in the bicarbonate.   This ion frequently increased to twice
blood level.

The gall-bladder wall gave evidence of damage much earlier when
solutions of calcium chloride were used.   When $CaCl_2$, 50 m.Eq. per liter
was used, increase of the calcium concentration in the solution did not
take place beyond five hours and hemorrhage into the solution occurred
within eighteen hours.

The calcium concentration always fell much more rapidly when the
gall-bladder had been damaged.   In those gall bladders into which fluid
was flowing the fluid entering the gall bladder had a calcium concentration
varying from 2.5 to 5.0 m.Eq. per liter.   The amount of fluid entering
the gall bladder in a 16 hour period was as high as 33 cc.   The fluid was
slightly viscid, clear, and contained varying quantities of stringy mucus.

Histology.   The gall bladder, when removed, was filled with formalin
after tying off the cystic duct.   Thus, a fully distended gall bladder was
fixed and it was possible to cut circular sections of the entire gall-bladder
wall.   Sections were stained with hematoxylin and eosin and with silver
nitrate (von Kossa method).

Calcium was found in the submucosa of every gall bladder which had
stopped absorbing water.   In the two instances when calcium chloride
was used, calcium could be demonstrated in the submucosa 24 hours after
the beginning of the absorption studies.   In the gall bladder in which
calcium lactate had been used, the calcium deposits were not observed for
several days after the beginning of the experiment.   As the calcium
deposit increased some calcium was found in the muscularis, although the
amount was small.   At this time, ulceration of the mucosa was observed

and calcium plaques appeared on the surface of the inner wall of the gall bladder. Associated with these more extensive changes was a marked scarring of the gall-bladder wall from fibrosis and in many instances the villi of the gall bladder disappeared leaving a surface covered with at most a single layer of low cuboidal epithelial cells (fig. 1).

DISCUSSION. The studies reported in this paper have been concerned solely with the absorption of calcium solutions from the bile-free gall bladder, the blood vessels and major lymphatics of which were not damaged. Our results would indicate that the gall bladder can concentrate calcium. The variation in calcium absorption in the day and night periods during the first few days of study is striking. The explanation for this is not obvious. Similar findings have not been previously reported. It
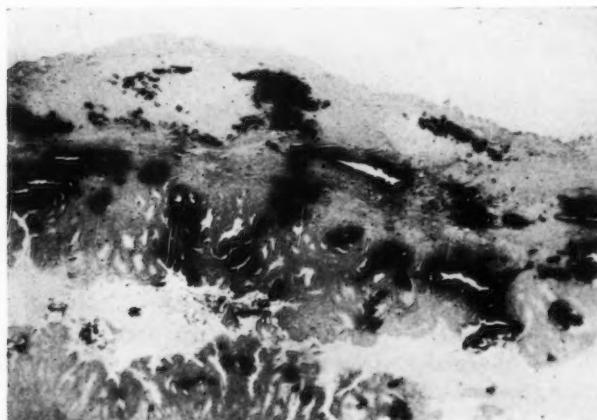
Fig. 1. Section stained by von Kossa method. Gall bladder removed 11 days after repeated introduction of calcium lactate solution containing 52 m.Eq. per liter.

apparently has a direct relationship to activity and not to feeding. When the animals were kept awake through the night the behavior during the night period resembled that of a day period.

The older analytic studies of Hammarsten (1906) did not include calcium studies. The literature on calcium absorption from the gall bladder is scanty. Drury (1924) showed that the calcium concentration of bladder bile was greater than the calcium concentration of liver bile. Furthermore, he inferred that calcium was absorbed by comparing the changes in calcium and bilirubin concentration in liver and gall-bladder bile.

Mirvish, Sacks and Schrire (1930) in a series of short acute experiments in anesthetized or decerebrate dogs, and using solutions of calcium chloride report in the main a reduction of the calcium concentration when no bile

was present, but an occasional increase when bile was present. These experiments can be criticised from more than one angle. Anesthetized animals are not satisfactory for this type of experiment. In our experiments calcium solutions decreased in concentration when anesthesia was continued and increased in concentration during the day period after recovery. Furthermore, puncture of the gall-bladder wall damages it and may change the rate of absorption. Important also is the fact that these investigators used calcium chloride which, as we have shown, causes early damage to the gall-bladder wall. Since histological studies were not reported we may suspect that had these been done the gall bladder would have shown changes similar to those which we have reported.

It might be supposed that calcium absorption from the bile-free gall bladder is not necessarily the same as that which occurs when the calcium is in solution in bile. Comparisons of fistula and gall-bladder bile (Drury, 1924) suggest, however, that the changes are in the same direction as long as the gall-bladder wall remains normal. Determinations of the calcium content of the liver and gall-bladder bile of the dog done in this laboratory but not reported, also indicate this.

Recently Andrews and Hrdina (1931) have analyzed gall-bladder and liver bile and have confirmed these findings. They, however, found a decrease in the concentration of calcium in gall-bladder bile many days after ligation of the cystic duct. Even then, however, the concentration was higher than that of fistula bile. Two criticisms may be made in connection with these experiments. These authors punctured the gall-bladder wall, and secondly, ligated the cystic duct. Ligation causes changes in the gall-bladder wall since it is not possible to ligate the cystic duct routinely without damage to major lymphatic vessels, so that the fluid removed 56 days after operation did not come from a normal gall bladder.

The determination of normality in our gall-bladder preparations is difficult. When sodium chloride was used, the rate and direction of water transfer was used as an indicator of this. In the sodium chloride experiments chloride was absorbed even though the rate of water absorption was decreased or the direction of transfer reversed. In the calcium experiments the decrease in calcium concentration during day periods was the first evidence noted of a change in the gall-bladder wall. Changes in the rate or direction of water transfer occurred at a later period.

SUMMARY

When solutions containing 3 to 100 m.Eq. per liter of calcium lactate were introduced into the normal bile-free gall bladder, the calcium concentrations increased during the day when the animals were active and decreased during the night or rest period. The lactate concentrations

did not show this diurnal variation to the same extent. There was a diurnal variation in the rate of water absorption from the gall bladder, the rate being greater during the day or active period.

Regardless of the concentration of the solution used total osmolar concentration tended to approach that of serum. The additional base was made up largely of sodium, and the additional anion largely of chloride and bicarbonate with traces of carbonate and phosphate.

From these studies we would conclude that calcium is not secreted into the gall bladder when the gall bladder is normal, but that a small amount may be secreted when the gall bladder is damaged.

After repeated introduction of calcium lactate there was a change in the behavior of the gall-bladder wall with respect to calcium, the calcium being absorbed at a greater rate than water even during the day periods, so that the concentration of calcium in the solutions decreased. When this occurred the total ion concentration increased, the anion increase resulting in large part from an increase in bicarbonate.

Microscopic studies of the gall bladder which had been damaged by repeated injections of calcium lactate or chloride showed deposits of calcium in the gall-bladder wall.

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STUDIES OF GALL-BLADDER FUNCTION[1]

VI. The Absorption of Bile Salts and Cholesterol from the Bile-Free Gall Bladder

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Since the discovery of the bile salts by Strecker (1848) considerable interest has been manifested in the rôle which they play in physiological and pathological processes. However, when one compares the knowledge of the physiology of bile acids with that of bile pigment it is indeed scant.

Although cholesterol metabolism has attracted considerable attention for many years and the rôle which it plays in the pathologic physiology of biliary tract disease has been widely investigated, there are no available quantitative data on cholesterol absorption from the gall bladder.[3] Lichtwitz (1929) suggested that cholesterol combined with desoxychlolic acid may be absorbed from the gall bladder, but no data are available to either refute or substantiate this statement. Previously Lichtwitz (1914) had suggested a double source of the bile cholesterol, from the liver cell and from the gall-bladder wall. The controversy between Naunyn (1896, 1923) Aschoff (1924), and Aschoff and Bacmeister (1909) as to whether the cholesterol is absorbed from or secreted into the gall-bladder bile has not been settled.

In an attempt to clarify certain of the perplexing problems in relation to bile salts and cholesterol and as part of a study of the fate of certain constituents of bile in the gall bladder, we have studied the absorption of bile salts and cholesterol from the bile-free gall bladder of the unanesthetized dog.

METHOD. The method of preparing the animals used in these experiments was the same as that reported in a previous paper (Ravdin, Johnston, Austin and Riegel, 1932). The bile salts (Eastman Kodak Co.) were either dissolved in water or phosphate buffered solutions, and were deter-

[3] As this paper is going to press two papers (Elman and Taussig (1931) and Elman and Graham (1932)) dealing with this subject has been published. These authors state that the gall bladder secretes cholesterol.

mined by the method of Gregory and Pascoe (1929).   When the standard was made up in the same medium as the solution introduced into the gall bladder, and when the volume of the standard used was the same as the volume of the unknown sample, the error of the method was about 5 per cent.

The cholesterol (Merck) suspension was prepared by a modification of the method described by Porges and Neubauer (1907).   Cholesterol was

TABLE 1

Dog 593, weight, 12 kgm.   Sodium glycocholate (not buffered)

TIME	CONCEN-TRATION IN	CONCEN-TRATION OUT	VOLUME IN	VOLUME OUT	VOLUME CHANGE	AMOUNT IN	AMOUNT OUT	AMOUNT CHANGE
hours	per cent	per cent	cc.	cc.	cc.	mgm.	mgm.	mgm.
2	0.460	0.840	14.0	4.5	−9.5	64	38	−26
2½	0.479	0.881	15.0	6.0	−9.0	72	53	−19
2	0.432	0.721	15.0	6.0	−9.0	65	43	−22

Blood in specimen 16 hours later
Total sodium glycocholate in = 201 mgm.
Total sodium glycocholate out = 134 mgm.
Difference = 33.3 per cent

TABLE 2

Bile salts

DOG	TIME	AMOUNT IN	AMOUNT OUT	AMOUNT LOST	DIFFERENCE
	hours	mgm.	mgm.	mgm.	per cent
354	2½	74	52	22	−29.8
357	5	96	89	7	−7.2
274	4¾	533	406	127	−23.8
392	5	207	177	30	−14.4
288	4½	200	167	33	−16.5
593	6½	201	134	67	−33.3
192	5	412	339	73	−17.7
					20.4 ±3.3

estimated by the method of Autenrieth and Funk as described by McMaster (1924), except that we used a solution of cholesterol in chloroform as a standard.   The error of determination of a known amount by this method in our hands has been about 10 per cent.   All estimations were done in at least duplicate.

RESULTS.   The gall bladders used in these experiments were normal unless otherwise indicated except for the last 55 hours of dog 882 (table 4). By normal we mean that water was absorbed at a rate comparable to that

obtained in the sodium chloride experiments during an active period. When bile salts were used any evidence of blood in the recovered sample was taken as an indication of damage. This constantly occurred within 24 hours after beginning the experiment.

A. Absorption of bile salts. Table 1 gives a typical experiment in which sodium glycocholate was used. Table 2 contains a summary of all the bile salt experiments. The results in all cases were in the same direction.

TABLE 3

Dog 573, weight, 14.5 kgm. Cholesterol

TIME	CONCEN-TRATION IN	CONCEN-TRATION OUT	VOLUME IN	VOLUME OUT	VOLUME CHANGE	AMOUNT IN	AMOUNT OUT	DIFFER-ENCE
hours	*per cent*	*per cent*	*cc.*	*cc.*	*cc.*	*mgm.*	*mgm.*	*mgm.*
$3\frac{1}{2}$	0.100	0.105	24.0	12.0	−12.0	24	15	−9
16	0.114	0.454	23.0	5.0	−18.0	26	31	+5
$4\frac{1}{2}$	0.108	0.114	15.0	5.0	−10.0	16	12	−4
3	0.109	0.159	16.0	5.0	−11.0	17	21	+4

Total cholesterol in = 83 mgm.
Total cholesterol out = 79 mgm.
Difference = 4.8 per cent.

TABLE 4

Cholesterol

DOG	AMOUNT IN	AMOUNT OUT	DIFFERENCE	
	mgm.	*mgm.*	*mgm.*	*per cent*
*474	120	112	−8	−6.6
*437	95	69	−26	−27.3
573	83	79	−4	−4.8
730	69	62	−7	−10.1
556	49	33	−16	−32.6
558	47	36	−11	−23.4
524	170	174	+4	+2.3
882	51	53	+2	+3.9
911	72	63	−9	−12.5

* Gall bladder not washed.

Three things constantly occurred: 1, the volume of fluid in the gall bladder decreased; 2, there was a decrease in the absolute amount of bile salt in the gall bladder; 3, the solution became more concentrated, indicating that water was leaving the gall bladder more rapidly than bile salt. With the exception of the first and sixth experiments in this table, the periods were approximately uniform. Since the per cent absorption in these two experiments was within the range of the remainder of the data, they have

been included in estimating the average absorption and the standard error of the mean.   The average absorption was 20.4 per cent ±3.3.

There was no appreciable difference in the per cent absorption of sodium taurocholate or sodium glycocholate.   Both salts caused intense inflammation of the gall-bladder wall and this was accompanied by hemorrhage into the gall bladder.   Histologic studies of the gall-bladder wall confirmed the gross findings.

B. Absorption of cholesterol.   Table 3 gives the results of a single experiment using a water suspension of cholesterol, and table 4 a summary of nine experiments.   As in the case of bile salts water passed through the wall of the gall bladder readily.   The concentration of cholesterol in the gall bladder increased as this occurred.   In the majority of instances varying amounts of the cholesterol were precipitated from the suspension. It was necessary to wash out this precipitated material at intervals from the gall bladder with physiologic salt solution and to add the estimated amount of cholesterol in these washings to the total.

The period over which these nine experiments were conducted varied from 7 to 105 hours with an average of 40 hours.   Favorable conditions for absorption or secretion of cholesterol existed in these experiments since the concentration of the suspension introduced varied from 0.042 per cent, which is below the average level found in dog's fistula bile to 0.506 per cent, which is above it.

Even after washing the gall bladder some cholesterol remained attached to the wall.   This could be seen in sections stained with Scharlach R, and probably accounts in part for the variability of our results.

In an attempt to ascertain how much cholesterol was in the gall-bladder wall, some of the gall bladders were analyzed.   Comparison of analyses of such gall bladders with the analyses of normal gall bladders shows them to contain slightly more cholesterol per gram of dried tissue than the normal, which would suggest that at least part of the cholesterol not recovered was not absorbed, but was adherent to the mucosa (table 5).   It would seem that little if any cholesterol was absorbed through the wall of the bile-free gall bladder in these experiments.

C. Cholesterol content of the gall bladder with hydrops.   Various investigators have differed as to whether cholesterol is present in the fluid of hydrops of the gall bladder.   We thought that a study of the fluid obtained from gall bladders which were pouring fluid into the lumen might throw some light on the question of absorption or secretion of cholesterol from the abnormal gall bladder.   We have analyzed the fluid obtained from four animals whose gall bladders were damaged to the extent that fluid was pouring into their lumen.   The condition was analogous to hydrops.   The fluid so obtained never exceeded 0.05 mgm. of cholesterol per cubic centimeter which is lower than the amount found in normal hepatic dog bile.

D. Bile salts and cholesterol. In another series of experiments a mixture of sodium taurocholate and a suspension of cholesterol was introduced into the gall bladder. The concentration of sodium taurocholate varied from 3 mgm. per cc. to 8.7 mgm. per cc., while the cholesterol varied from 0.3 mgm. per cc. to 1.7 mgm. per cc. The suspension of cholesterol was considerably more stable in the mixture. Precipitation of the cholesterol

TABLE 5

Analyses of dried gall bladders for cholesterol

	GALL BLADDER	WEIGHT	CHOLESTEROL	MGM./GM.
		grams	*mgm.*	
Normal	N 1	0.1495	1.5	10.0
	N 2	0.390	2.2	5.6
	N 3	0.260	4.4	17.0
	N 4	0.276	5.9	21.5
	N 5	0.422	7.9	18.7
	N 6	0.2015	1.6	8.1
	N 7	0.286	6.6	23.0
Experimental	911	0.078	2.9	37.4
	882	0.078	2.7	34.6
	987	0.720	15.9	22.1
	878	0.820	12.2	14.8

TABLE 6

Bile salt-cholesterol mixture

DOG	BILE SALT				CHOLESTEROL			
	Mgm. in	Mgm. out	Mgm. lost	Per cent total lost	Mgm. in	Mgm. out	Mgm. lost	Per cent total lost
602	266	175	91	34.0	66	53	13	19.6
603	330	186	144	43.6	96	82	14	14.5
497	987	920	67	6.8	151	124	27	17.8
560	303	206	97	32.0	78	74	4	5.1
719	423	327	96	22.6	132	128	4	3.0
606					59	51	8	13.5
987					97	83	14	15.0
878					109	97	12	11 0

from the mixture after placing it in the gall bladder rarely occurred and in the one or two instances in which it did, only a very small amount of cholesterol was found in the washings.

The experiments in this series covered as long as 190 hours. Every time a solution was introduced a sample of it was analyzed, even though it had been done at a previous time. The figures given in table 6 are summaries for the period of each experiment.

A very interesting aspect of these experiments was the longer period of time which elapsed before blood appeared in the gall-bladder solution. When the bile salts were used alone, blood frequently was present within five hours and at most twenty-four hours.   When a mixture of bile salts and cholesterol was used the shortest time necessary to cause hemorrhage was twenty-one hours and the longest over one hundred and ninety hours. It would appear that the presence of cholesterol prevented, to an extent, the inflammatory action of the bile salts.

In all experiments in this series, water was absorbed as was sodium taurocholate.   The average period of hours over which the simple bile salt experiments were conducted was four hours and forty-five minutes, while the average time for the mixture of bile salt and cholesterol was twenty-nine hours, or approximately six times as long.   The per cent absorption of bile salt per hour in the first series of experiments averaged 4.1; while the figure for the five mixture experiments where bile salts were estimated was 0.92 per cent.   This was reflected in the higher concentrations to which the bile salts rose in the mixture experiments.

The amount of cholesterol absorbed was indeed very small.   The figures in table 6 show that in three instances the amount that was lost was within the experimental error of the method.   Since in many of the gall bladders removed from these animals some cholesterol could be demonstrated in the gall-bladder wall, it is quite likely that little if any cholesterol was absorbed.   Even if some was absorbed, the amount over a long time interval is too small to be of any real significance.

E. Bile salt-cholesterol absorption from the damaged gall bladder.   We were unable to study bile salt absorption from the gall bladder, damaged as the result of simple bile salt introduction because of the presence of blood in the solutions.   We therefore used gall bladders which had been damaged either by infection or by the repeated use of hypertonic solutions. Water was either not absorbed or fluid entered the gall-bladder lumen, increasing the fluid contents of the gall bladder.

When into this type of preparation a bile salt-cholesterol mixture was introduced the results were entirely different from those obtained when a similar mixture was placed in the normal gall bladder.   The bile salt concentration decreased due to absorption of bile salt, dilution or both.

A very interesting observation in this type of preparation was the increase in the cholesterol content of the gall-bladder fluid.   In one experiment the excess cholesterol recovered in the solution was approximately 1 mgm. per cc., or 20 times that found in gall bladders which were simply pouring fluids in, but in which no bile salt-cholesterol mixture was present.

DISCUSSION.   The data on simple bile salt absorption are in accord with observations published by several investigators who have estimated the concentration of bile salts in fistula and gall-bladder bile.   Aldrich and

Bledsoe (1928) published results which would indicate a greater concentration of bile salts in the dog than in the human gall bladder. Ravdin, Morrison and Smyth (1929), using the Aldrich-Bledsoe method, reported a lower concentration of bile salts in human gall-bladder fistula and common duct fistula bile than these authors reported.

The gall bladders from which Ravdin, Morrison and Smyth (1929) obtained their fistula bile were diseased. It is interesting to note, however, that the concentration of bile salts which they found in gall-bladder fistula bile was lower than the concentration found in common duct fistula bile. It would appear that the diseased gall bladder causes a reduction of bile salt concentration. This observation is confirmed by our data on the bile salt concentration of a bile salt-cholesterol mixture after its introduction into the damaged gall bladder.

The reduction of bile salt concentration in the damaged gall bladder has also been observed by Newman (1931) and Andrews, Schoenheimer and Hrdina (1931). The latter authors have also conducted experiments on the dog but these have not been reported except in a preliminary report (1931a).

Rosenthal and Licht (1928) studied the absorption of bile salts after tying the cystic duct of the dog. Through a puncture hole they irrigated the gall bladder until the fluid returned clear. They then injected a known amount of bile salt and closed the puncture hole by suture. In the course of two to ten days they found that 12 to 87 per cent of the bile salts had been absorbed. Rosenthal and Licht do not report the amount of fluid in the gall bladder at the conclusion of their experiments so that one cannot determine whether water was being constantly absorbed. In every normal experiment reported in our series, water was being absorbed throughout the period of the experiment. From this point of view at least the gall bladder was still functioning normally. In the gall bladder of experimental cholecystitis Rosenthal and Licht found even greater bile salt absorption.

The method which they employed, ligation of the cystic duct and puncture of the gall bladder, however, is not without criticism. It is impossible to ligate the cystic duct routinely without injury to major lymphatics and blood vessels. After cystic duct ligation the gall bladder cannot be considered normal. Its wall becomes thickened and infection frequently occurs. This method, which has been used in many of the studies of gall-bladder absorption, impairs the validity of the experimental observations if the investigators report their results as obtained from a normal viscus. Any trauma to the gall bladder, such as puncture, has been known to increase the rate of absorption (Winkenwerder, 1930). The insertion of a needle and subsequent ligature of the opening is followed by a marked local change in the gall-bladder wall so that it is not possible to consider the organ as normal after this procedure.

No mention was made by them of blood in the fluid a number of hours after the introduction of the bile salts.   In the concentrations which we used, the fluid constantly became bloody after at most twenty-four hours. The purity of the bile salts apparently made no difference since in one experiment we used a recrystallized salt and blood was present in the fluid within eighteen hours after its introduction.

The studies of Hammarsten (1929) and others have shown that the concentration of cholesterol in gall-bladder bile is greater than that of fistula bile.   The concentration factor for mucin and pigment calculated from Hammersten's data is 8.85, while that for cholesterol is 7.5.   He did not do quantitative studies on the amount of bile which entered the gall bladder during these studies.   Our data from the use of cholesterol suspensions are indicative of little if any cholesterol absorption and thus are similar to those of Hammarsten.

Aschoff (1906) suggested that the epithelium of the gall bladder absorbed a cholesterol-ester-neutral fat mixture.   The ester he thought, was split and the chlolesterol returned to the gall-bladder bile, while the fat was transported.   There has been no experimental confirmation of this theory.

Lichtwitz (1929) thought it possible that cholesterol in an additive combination with desoxycholic acid could be absorbed.   In our experiments with a mixture of bile salts and cholesterol there is a suggestion that a small amount of cholesterol may be absorbed but the amount is indeed very small.

Iwanaga (1923) found that when cholesterol in oil was placed in the gall bladder, there was evidence of the absorption of oil but little or no evidence that cholesterol was absorbed.

There is no indication in our data that cholesterol in significant amounts is absorbed from the normal gall bladder in a twenty-four to forty hour period.

There has been considerable controversy as to whether cholesterol is absorbed or secreted by the gall-bladder wall.   Favorable conditions for absorption or secretion of cholesterol in the normal gall bladder existed in these experiments since the concentrations varied in the different suspensions from 0.042 per cent, which is below the average level found in bile, to 0.494 per cent, which is above it.   We may be criticised for using a suspension of cholesterol without any definite knowledge that cholesterol in bile is present as a colloid.   We were unable, however, to find a better method for its use which would not at the same time cause gall-bladder damage.

In the normal gall bladder we have no intimation from our data that cholesterol is secreted by the gall-bladder mucosa.   When the gall bladder is damaged the fluid pouring into the gall bladder carries with it cholesterol.

This may come from the epithelial cells of the gall-bladder mucosa as Naunyn (1896) suggested. In simple hydrops the amount is indeed small, not exceeding 5 mgm. per 100 cc. of hydropic fluid. However, in the presence of bile salt and cholesterol, we have evidence that a greater amount may be secreted into the gall bladder.

Illingworth (1929), from two experiments on cats, found a loss from the gall bladder in a period of five days of more than 50 per cent of the cholesterol introduced. He concludes, however, that cholesterol is only absorbed when it is present in excess. The total amount lost—18 and 21 mgm. respectively—over a period of five days is indeed very small.

A very careful distinction should be made by investigators in this field as to the type of gall bladder with which they are working. It is our belief that an increase in the total cholesterol content in a preparation is associated with damage to the viscus.

SUMMARY

When a solution of bile salts is introduced into the normal gall bladder there occurs: 1, absorption of water; 2, absorption of bile salts; 3, concentration of the solution, indicating a greater absorption of water than of bile salts.

When a suspension of cholesterol in water is introduced into the normal gall bladder there occurs: 1, absorption of water; 2, concentration of the suspension; 3, little or no absorption of cholesterol.

When a suspension of cholesterol in a solution of sodium taurocholate is introduced into the normal gall bladder there occurs: 1, absorption of water; 2, absorption of bile salt; 3, concentration of solution; 4, little or no absorption of cholesterol.

Fluid from dogs' gall bladder with hydrops contained less than 0.05 mgm. of cholesterol per cubic centimeter.

When a bile salt-cholesterol mixture is introduced into the damaged gall bladder there occurs: 1, absorption of bile salt; 2, decrease in concentration of solution; 3, increase in cholesterol content of mixture.

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THE RELATION OF THE SODIUM, POTASSIUM, AND CALCIUM IONS TO THE HEART RHYTHMICITY

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While all observers agree that a properly balanced mixture of sodium, potassium, and calcium salts is essential for the maintenance of the heart's activity, there is lack of agreement in regard to the special rôle played by each of the cations in such a mixture. The initiation and maintenance of the spontaneous rhythmic contractions have been attributed chiefly to the sodium ion (Loeb, 1900; Lingle, 1900), or to the sodium and calcium ions together (Howell, 1901). On the other hand, the electrical and chemical but not the mechanical changes have been referred chiefly to the sodium ion (Locke, 1907), while the mechanical contraction has been related especially to the action of the calcium ion (Ringer, 1884; Locke, 1907; Mines, 1913; Clark, 1913; et al). Potassium has in general (except for Zwaardemaker) been assumed to be important chiefly as an antagonist to calcium (Ringer, Howell, etc.). Some experimenters have considered that in addition to these ions other substances of equal or greater importance must be present for automatic rhythmicity of the heart muscle, such as the hydrogen and hydroxyl ions in proper concentration (Mines, 1913; Andrus and Carter, 1924; and Dale and Thacker, 1914); certain organic substances such as lipoids (Clark, 1913); an organic heart hormone (Demoor, 1929; Haberlandt, 1927; and Zwaardemaker, 1928, the latter considering potassium essential for its activation); or the products of metabolism (Langendorff, 1902; Engelmann, 1897; and Martin, 1906).

In much of the earlier work the influence of the sodium, potassium, and calcium ions was estimated only in terms of the mechanical response of the cardiac muscle. The work of Locke (1907), Mines (1913), Hogben (1925), and others, however, has shown that in the absence of visible contractions rhythmic electrical variations may still be present, indicating the formation and conduction of an inner stimulus. This has been criticized by Einthoven (1924–25), de Jongh (1926), and Arbeiter (1921) on the basis that the mechanisms for recording the mechanical contractions were not equal in sensitiveness to those recording the electrical variations. It was decided, therefore, to reinvestigate the effect of the sodium, potassium,

and calcium ions on heart rhythmicity paying attention to both the mechanical and electrical response, and utilizing a new device for mechanical registration of greater delicacy than any hitherto employed.

METHODS. The majority of the experiments were made upon the terrapin sinus. This tissue is particularly suited for studying the effect of the various solutions on the heart beat, first, because it consists mainly or entirely of sinus musculature, which exhibits the property of automatic rhythmicity to the highest degree, and second, because the walls are thin thus allowing the perfusing solution to come into direct contact with a larger proportion of cells. It should, therefore, yield more significant results than experiments on the ventricle which consists of true cardiac muscle tissue as well as conducting tissue and in which, owing to the thickness of the wall, the internal cells are not in free communication with the perfusate.

Because of the existence of a partial septum between the right and left portions of the terrapin sinus, only the right portion was isolated and removed for experimentation. The perfusion fluid was introduced through a cannula in the right precaval vein and drained through the postcaval, the solution being supplied from a Marriotte flask. During the taking of the records, by closing off the two cannulas, the sinus could be maintained at a moderate degree of distention without collapse or introduction of fluid. This was of very great importance because during perfusion, as each bubble of air entered the Marriotte flask, mechanical and electrical deflections resulted which appeared as weak rhythmical electrical and mechanical beats. In two experiments the whole terrapin heart excepting the left auricle and left portion of the sinus was used, the perfusion fluid being introduced through the right precaval vein and drained through extensive cuts in the ventricle. In one experiment the whole frog heart was used, the perfusate being introduced through the ascending vena cava and drained through the bulbus arteriosus.

The variations in the electrical potential were recorded by means of a Hindle electrocardiograph. In the majority of the experiments an extremely loose string was used so that, with the sinus in the circuit, the introduction of one millivolt produced a deflection of ten to fifteen centimeters. Nonpolarizable silver chloride electrodes were used, one being placed on the sinus in close proximity to the mechanical lever, and the other on a small piece of indifferent tissue left in contact with the sinus and killed by heating. The mechanical contractions were recorded simultaneously with the electrical variations on the same film by means of a very delicate apparatus devised by Max (1931), the movements of the sinus being magnified 1400 times.

Isotonic solutions of recrystallized chemicals were always freely perfused through the tissue. The outside of the tissue was frequently moistened

and in some experiments, notably those in which the whole heart was used, a continuous stream of the perfusate was allowed to run over the external surface as well as through the tissue chambers. The hydrogen ion concentration of all solutions, unless otherwise stated, was adjusted to a pH of 7.1 with sodium bicarbonate or sodium hydroxide. The sinus was always perfused with Ringer's solution (NaCl 0.70 per cent, KCl 0.040 per cent, CaCl$_2$ 0.025 per cent) during its preparation and for a short time at the beginning of the experiment until normal electro- and mechanograms were obtained. It was likewise perfused at the end of each experiment to determine if the sinus was still surviving or if the test solution had rendered it incapable of recovery.

DATA AND RESULTS: PART I. EXPERIMENTS ON THE TERRAPIN SINUS. The effect of each of the three ions, sodium, potassium, and calcium, on the electrical and mechanical phenomena of the terrapin sinus was studied in general along three lines: first, perfusing the sinus with an ion in isotonic solution until all activity ceased and recording the changes which occurred; second, testing the ability of the ion to produce a recovery of the electrical and mechanical variations in a sinus previously perfused to quiescence with an isotonic solution of a non-electrolyte, usually dextrose, or perfused to quiescence with an isotonic solution containing one or both of the other ions; and third, removing the ion from the normal Ringer's mixture both in perfusing the sinus and in recovering the sinus. When a solution, perfused through a sinus for 1 to 1½ hours, failed to recover any rhythmic activity in a sinus previously perfused to exhaustion, it was considered ineffective. The sinus was then perfused either with a solution of another electrolyte or with Ringer's solution.

As a basis for the sodium, potassium, and calcium experiments the result of perfusing the sinus with Ringer-Locke's solution (combining the three ions in proper proportion) and of perfusing the sinus with an isotonic dextrose solution (lacking these three ions) is first presented. The effect of prolonged perfusion of the terrapin sinus on Ringer's or Ringer-Locke's solution is well known. The electrical and mechanical variations decreased in amplitude very gradually. At the end of 7 to 10 hours there was in some cases a noticeable decrease from the normal, while in others the difference was slight. There was usually a slight decrease in rate. When the sinus was perfused with an oxygenated isotonic dextrose solution free from all electrolytes, the magnitude of the electrical and usually also of the mechanical beat increased greatly after about one-half hour of perfusion. This was possibly due to the non-conducting character of the perfusate, so far, at least, as the electrical variation is concerned. This lasted for only a short period, after which the electrical and mechanical beats decreased gradually in extent, and finally disappeared simultaneously. The duration of the beat is shown in table 1.

1. *The relation of the sodium ion to the terrapin sinus. Perfusion with sodium:* The terrapin sinus was perfused with a sodium chloride solution (0.7 per cent or 0.2 per cent made isotonic with dextrose) in four experiments. The electrical and mechanical activity gradually decreased in amplitude, the latter decreasing usually more rapidly than the former. The duration of the beats and the rate are given in table 1. In two experiments small electrical variations survived the mechanical contractions by $\frac{3}{4}$ and $\frac{1}{2}$ hour, as illustrated by record 1. That the sinus was capable of

TABLE 1

Showing the survival time of the rhythmicity of the terrapin sinus when perfused with various solutions

PERFUSION SOLUTION	NO. OF EXPERI- MENTS	RATE IN BEATS/MIN.	TYPE OF ACTIVITY	LENGTH OF TIME SINUS ACTIVITY WAS MAINTAINED	
				Extremes in different experiments	Average (expressed to nearest quarter hour)
				hours	*hours*
CaCl$_2$	3	slowing to 3/min.	mechanical / electrical	$1\frac{3}{4}$–2	$1\frac{3}{4}$
Dextrose	27	slowing to 3/min.	mechanical / electrical	$\frac{3}{4}$–$6\frac{1}{2}$	$2\frac{1}{4}$
NaCl + CaCl$_2$	1		mechanical / electrical		$2\frac{1}{2}$
NaCl	4	10/min.	mechanical / electrical	$\frac{1}{2}$–8 / $\frac{1}{2}$–$8\frac{1}{2}$	$3\frac{1}{4}$ / $3\frac{1}{4}$+
NaCl + KCl	3	10/min.	mechanical / electrical	2–$9\frac{3}{4}$ / 2+–$10\frac{1}{2}$	$5\frac{1}{2}$ / $5\frac{1}{2}$+
Ringer's solution		15/min.	mechanical / electrical	beats only slightly less than normal after 7–10 hours perfusion	

further activity after perfusion to exhaustion on sodium chloride was proved by the rapid and good recovery effected by perfusion with Ringer's solution.

Recovery on sodium: The ability of the sinus to recover its rhythmicity by perfusion with NaCl, 0.2 per cent or 0.7 per cent, following cessation of the electrical and mechanical variations as a result of perfusion with dextrose, was tested in eighteen experiments. Rhythmicity was recovered in nine of these experiments, both the electrical and mechanical phenomena

reappearing in each case. The extent of recovery was usually small (record 2) although in some experiments it was quite good (record 3); and the electrical recovery was usually, although not invariably, somewhat better than the mechanical. In spite of the marked slowing of the rhythm on the dextrose solutions, the rate of the beat recovered with sodium chloride was almost as rapid as the original rate of the sinus on Ringer's solution and was very regular. The length of time necessary for perfusion

TABLE 2

Showing the ability of the sinus to recover its rhythmicity on the various solutions follow-ing previous perfusion to quiescence

RECOVERY SOLUTION	PREVIOUS EXHAUSTING SOLUTION	NO. EXP.	NO. RECOVERIES	TIME NECESSARY FOR RECOVERY	DURATION OF RECOV-ERED RHYTHMS
NaCl (*pH 7.1*)	Dextrose	18	9	15–60 min. avg. 27 min.	mech. $\frac{3}{4}$–$2\frac{1}{2}$ hours elect. $\frac{3}{4}$–$3\frac{1}{2}$ hours
NaCl (*pH 8*)	Dextrose	2	1	60 min.	mech. elect. $\}$ $1\frac{3}{4}$ hours
NaCl (*pH 7.1*)	Urea	2	1	50 min.	elect. 20 min.
NaCl (*pH 7.1*)	CaCl$_2$ 0.025%	3	3	3–7 min.	mech. 50 min.– $1\frac{3}{4}$ hrs. elect. 50 + min.– $1\frac{3}{4}$ hrs.
NaCl (*pH 7.1*)	CaCl$_2$ 0.010%	1	1	35 min.	mech. elect. $\}$ 2 hours
NaCl (*pH 7.1*)	NaCl + CaCl$_2$		no recovery		
CaCl$_2$ (*pH 7.1*)	Dextrose		no recovery		
KCl (*pH 7.1*)	Dextrose		no recovery		
CaCl$_2$ + KCl (*pH 7.1*)	Dextrose		no recovery		

to recovery is given in table 2 together with the duration of the recovered rhythms. The electrical variation again survived the mechanical con-tractions by 1 hour and $1\frac{3}{4}$ hours in two experiments. Electrical and mechanical activity was again recovered by perfusion with Ringer's solution. The ability of the sinus to recover on 0.2 per cent NaCl with a pH 8, following perfusion to exhaustion on dextrose, was tested in two experiments with small electrical and mechanical recovery in one case

(see table 2). The results are within the limits of those described above in which the pH of the perfusate was 7.1. In two experiments the sinus was perfused to exhaustion on urea (14 gm. per liter) in place of dextrose. The electrical beat remained much stronger than the mechanical beat although both disappeared simultaneously. In only one experiment was recovery obtained and then electrical variations only were temporarily recovered by perfusion with 0.7 per cent NaCl. The inability of the sinus to recover its rhythmicity on a sodium chloride solution following exhaustion on sodium chloride plus calcium chloride (NaCl 0.7 per cent plus CaCl₂ 0.025 per cent or 0.010 per cent) was demonstrated in five experiments. Ringer's solution produced slight electrical and mechanical recovery in only two of these experiments. On the contrary a 0.7 per cent NaCl solution produced immediate recovery of the electrical and mechanical beats in three experiments following perfusion to exhaustion on a solution of calcium chloride (0.025 per cent) and a slightly delayed recovery when the concentration of the calcium chloride was 0.01 per cent. The recovered mechanical beats were usually proportionately better than the electrical deflections but were small. The time necessary for recovery and the duration of the recovered rhythms are given in table 2. Activity was again recovered on Ringer's solution.

Lack of sodium: The inability of the sinus to recover from dextrose exhaustion on a solution lacking in sodium, and consisting only of calcium chloride (0.025 per cent) plus potassium chloride (0.040 per cent) made isotonic with dextrose, was demonstrated in three experiments. This result confirms some unpublished results obtained by Howell in 1930.

2. *The relation of the calcium ion to the terrapin sinus. Perfusion with calcium:* In perfusing the sinus with a calcium chloride solution, 0.025 per cent or 0.010 per cent made isotonic with dextrose, the electrical and mechanical deflections responded in the same manner as when dextrose alone was perfused; the variations remained very good for some time but became slower in rate and then gradually diminished in amplitude to simultaneous disappearance. The length of time necessary for perfusion to exhaustion on calcium chloride is given in table 1. The electrical and mechanical rhythms were rapidly recovered on 0.7 per cent NaCl in all cases (see above under sodium recovery experiments).

Recovery on calcium: The inability of the sinus to recover on calcium alone following exhaustion on dextrose was demonstrated in several experiments. Further perfusion with a solution containing sodium and calcium was likewise ineffective although slight recovery was obtained on Ringer's solution.

Lack of calcium: The effect of perfusing the sinus with a calcium-free Ringer's solution (i.e., a solution of sodium chloride plus potassium chloride in the proportions existing in Ringer's solution) was studied in three experi-

ments. The mechanical beats decreased rapidly at first, soon becoming very small, a condition which then persisted until they disappeared completely. The electrical deflections decreased only gradually, and in each case a definite electrical variation was observable after all traces of a mechanical beat had disappeared, as illustrated by record 4. The duration of the sinus rhythm is given in table 1. In only one experiment was the perfusion continued until the electrical variation ceased, which occurred within $\frac{3}{4}$ hour after the disappearance of the mechanical contractions. The sinus beat was, in each case, recovered by perfusion with Ringer's solution (which involved the addition of calcium only). The efficacy of a sodium plus potassium solution to produce recovery in a sinus, which had been perfused to exhaustion on dextrose and which had not been recovered by potassium chloride, was tried without success, although Ringer's solution caused a rapid recovery.

Record 1. Electro- (above) and mechano- (below) grams of a terrapin sinus which had been perfused for $2\frac{1}{2}$ hours with a 0.2 per cent NaCl solution made isotonic with dextrose. Note the survival of the electrical variations but complete lack of mechanical contractions. (Sensitivity of galvanometer 10 cm./mv.)

Record 2. Electro- (above) and mechano- (below) grams of a terrapin sinus recovered by perfusion with a 0.2 per cent NaCl solution made isotonic with dextrose, following perfusion to quiescence with dextrose. (Sensitivity of galvanometer 2 cm./mv.)

Record 3. Electro- (above) and mechano- (below) grams of a terrapin sinus recovered by perfusion with a 0.7 per cent NaCl solution, following perfusion to quiescence with dextrose solution. (Sensitivity of galvanometer 20 cm./mv.)

Record 4. Electro- (above) and mechano- (below) grams of a terrapin sinus which had been perfused for $5\frac{3}{4}$ hours with a solution of NaCl 0.7 per cent plus KCl 0.04 per cent. Note the survival of the electrical variations but complete lack of mechanical contractions. (Sensitivity of galvanometer 6.5 cm./mv.)

Record 5. Electro- (above) and mechano- (below) grams of the terrapin ventricle which had been perfused for 12 hours with a 0.7 per cent NaCl solution. Note the survival of the electrical variations but complete lack of mechanical contractions. (Sensitivity of galvanometer 10 cm./mv.)

Record 6. Electro- (above) and mechano- (below) grams of the terrapin ventricle which had been perfused for $12\frac{3}{4}$ hours with a solution of NaCl 0.7 per cent plus $CaCl_2$ 0.01 per cent. Note the large mechanical contractions as well as the electrical variations. (Sensitivity of galvanometer 10 cm./mv.)

Record 7. Electro- (above) and mechano- (below) grams of the frog auricle recovered by perfusion with 0.7 per cent NaCl following perfusion to quiescence with a solution of NaCl 0.7 per cent plus $CaCl_2$ 0.0125 per cent. Note the mechanical contractions accompanying the rhythmic electrical variations. (Sensitivity of galvanometer 25 cm./mv.)

Record 8. Electro- (above) and mechano- (below) grams of the frog ventricle recovered by perfusion with 0.7 per cent NaCl following perfusion to quiescence with a solution of NaCl 0.7 per cent plus $CaCl_2$ 0.0125 per cent. Note the rhythmic electrical variations and the lack of mechanical contractions. (Sensitivity of galvanometer 15 cm./mv.)
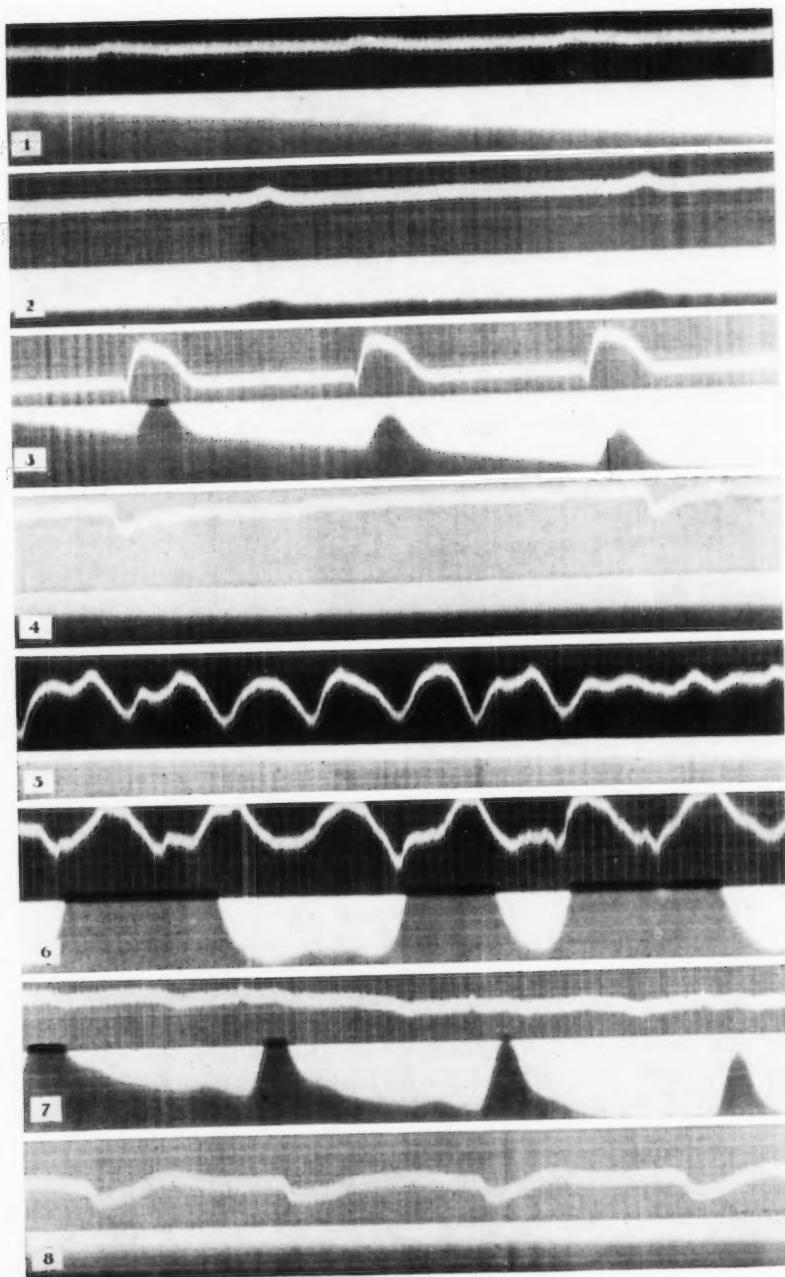
Records 1–8

3. *The relation of the potassium ion to the terrapin sinus. Recovery on potassium:* No experiments were performed in which the fresh terrapin sinus was perfused with a potassium solution. The inability of the sinus to recover its rhythmicity on a potassium solution (0.04 per cent made isotonic with dextrose) following exhaustion on dextrose was, however, demonstrated in several experiments.

Lack of potassium: The effect of a Ringer's solution lacking only in potassium and consisting of 0.7 per cent NaCl plus 0.025 per cent CaCl₂ was studied on one terrapin sinus. The electrical and mechanical deflections decreased together and disappeared simultaneously (see table 1). Recovery on 0.7 per cent NaCl was attempted without success. In three other experiments the sinus was perfused to exhaustion on dextrose and recovered with 0.7 per cent NaCl before being perfused with a sodium plus calcium solution (NaCl 0.7 per cent plus 0.025 per cent or 0.010 per cent CaCl₂). In two of these cases slight temporary improvement in the mechanical contractions occurred when calcium was added to the solution. The electrical and mechanical beats soon, however, disappeared simultaneously. Recovery was attempted with 0.7 per cent NaCl without success followed by Ringer's mixture with recovery in only one case. The inability of the sinus to recover its rhythmicity on a potassium free Ringer's solution was attempted without success after exhaustion on dextrose and failure to recover on calcium chloride and again after exhaustion on 0.7 per cent NaCl. Ringer's solution, however, produced recovery in each case.

PART II. EXPERIMENTS ON THE TERRAPIN AND FROG HEARTS. 1. *The effect of sodium chloride and sodium chloride plus calcium chloride on the terrapin heart.* In order to determine if the results described for the sinus were similarly true for the whole terrapin heart, two terrapin hearts were perfused, one with a 0.7 per cent NaCl solution and the other with a sodium plus calcium solution (0.7 per cent NaCl plus 0.01 per cent CaCl₂). When sodium was the only electrolyte in the perfusion solution the mechanical contractions decreased rapidly at first and then persisted as faint beats for some time. At the end of twelve hours, however, all mechanical contractions had ceased in the auricle and ventricle (record 5 taken from ventricle). During this time the electrical variations decreased somewhat in amplitude in the ventricle and markedly in the auricle and became extremely irregular in both chambers. On the contrary, during similar perfusion with sodium plus calcium for twelve hours, the mechanical contractions of the auricle and ventricle remained excellent in amplitude although they became irregular in form and rate (see record 6 from ventricle). The electrical variations were similarly affected by perfusion with sodium plus calcium as with sodium alone, as seen by comparing records 5 and 6. The decrease in amplitude and onset of irregularity in

the electrical deflections appeared earliest and to a greater extent in the sinus, next in the auricle and lastly in least measure in the ventricle. After 13½ hours the perfusion was stopped in each case and the sinuses were immersed in the respective solutions for 8 to 10 hours. The same condition resulted in both experiments, complete cessation of all activity except slight and irregular electrical movements in the ventricles. Perfusion with Ringer's solution produced a more rapid recovery of the ventricular electrical deflections following sodium alone, but the recovery, although delayed, was equally as good after sodium plus calcium. The mechanical contractions recovered in the ventricles were better after sodium plus calcium than after sodium alone.

2. *The effect of sodium chloride and sodium chloride plus calcium chloride on the frog heart.* In order to compare the terrapin and frog hearts in their response to the sodium and calcium ions, a frog heart was perfused as in the above experiment with a solution of sodium plus calcium (NaCl 0.7 per cent plus $CaCl_2$ 0.0125 per cent). At the end of 2¾ hours the electrical variation and the mechanical contractions had ceased in the sinus, auricle, and ventricle. The heart was then perfused with 0.7 per cent NaCl. At the end of 10 minutes small electrical and mechanical beats had returned in the auricle (see record 7), while only electrical variations of good amplitude appeared in the ventricle, as seen in record 8. The heart was then perfused again with sodium chloride plus calcium chloride. After 10 minutes the auricle and ventricle showed neither electrical nor mechanical deflections. When again perfused with sodium chloride for 30 minutes the mechanical and electrical deflections reappeared in the auricle, while, as before, only the electrical variations returned in the ventricle. A record of the sinus showed some irregular electrical variations but no mechanical contractions. This experiment confirmed some unpublished work of Howell and Max.

DISCUSSION. Analysis of the results emphasizes the importance of the sodium ion as the ion most essential to the initiation of the sinus rhythmicity. Perfusion with sodium chloride produced rhythmic electrical and mechanical activity in sinus tissue, previously made quiescent by perfusion with dextrose or calcium, and electrical recovery after exhaustive perfusion with urea, whereas calcium and potassium either alone or in combination were, on the contrary, never able to produce any evidence of recovery. In the frog heart, following perfusion to quiescence on sodium plus calcium, perfusion with sodium chloride initiated electrical and mechanical activity in the auricle and electrical activity alone in the ventricle. The ability of sodium to restore and maintain for a short while small mechanical beats in the terrapin sinus is not in accord with Clark's and Mines' theory that calcium is essential for mechanical shortening, unless it is assumed that usable calcium is retained by the sinus during dextrose exhaustion and subsequent recovery on sodium.

That the sodium ion is also an important factor in the maintenance of a normal rhythm is seen by the fact that a sinus perfused with a solution containing sodium is able to continue activity for a longer time than when perfused with a solution of dextrose or calcium; and, secondly, that only when sodium is present in the solution is the rate of activity maintained fairly normally.   That sodium, unsupported by the calcium and potassium, cannot maintain continuous electrical rhythmicity is shown by the comparatively short duration of sinus activity on a sodium solution, alone or combined with either of these other ions, as compared with the activity on Ringer's solution, and by the fact that, after exhaustion on a sodium solution, the activity is rapidly restored by perfusion with Ringer's solution.

Clark, Bouchaert and Belehrader (1927), and others have expressed the view that reduction in the concentration of sodium chloride from 0.65 per cent to 0.20 per cent is beneficial to muscular activity.   In these experiments two concentrations of sodium chloride were used for perfusing the sinus, 0.7 per cent NaCl and 0.2 per cent NaCl made isotonic with dextrose.   The sinus tissue reacted in an exactly similar manner to the two concentrations in respect to the following: the duration of the rhythm; the rate; the ability to recover rhythmicity after previous exhaustion on dextrose, and the length of time of perfusion before recovery; the extent, type, and duration of the recovered activity; and the survival of the electrical variations after cessation of the mechanical contractions.   Our results, therefore, fail to confirm the view that the weaker concentrations of sodium chloride favor the activity of the heart.

On the other hand, these experiments tend to confirm the opinion of previous investigators, Ringer, Howell, Locke, Mines, Clark, etc., with regard to the rôle of calcium, namely, that calcium is essential to the production of strong, functionally important, mechanical contractions. Whenever contractions were obtained on solutions free from calcium they were feeble and functionally ineffective.   This influence of calcium was shown in a striking way in the terrapin's heart.   When it was perfused with sodium alone, the mechanical contractions became steadily weaker and at the end of twelve hours the ventricle ceased to give any mechanical beats, although it still exhibited rhythmic electrical responses.   With the sodium chloride plus calcium chloride, on the contrary, mechanical contractions of the ventricle were excellent even after a perfusion of twelve hours.

As regards the influence of the calcium ion a remarkable difference was found between the frog and the terrapin heart.   In the frog heart, as was first shown in some unpublished experiments in this laboratory by Howell and Max, calcium tends to counteract the influence of sodium in causing impulse formation in the heart muscle.   A fresh frog's heart perfused with sodium plus calcium comes to complete rest in two to three hours,

giving neither a mechanical nor an electrical response. If then it is perfused with sodium chloride alone, rhythmical electrical responses occur in all chambers, and feeble mechanical contractions are exhibited by the sinus and auricles. Perfusion with sodium plus calcium rapidly inhibits all activity. Perfusion again with sodium alone recovers the activity as before, and further addition of calcium inhibits it. This result would seem to show that in the frog's heart sodium chloride alone suffices to bring about the rhythmic production and conduction of an inner stimulus as indicated by the electrical response, and that this activity of the sodium ion in the absence of potassium is counteracted or inhibited by the presence of calcium in physiological concentration. From these results it would appear that the calcium ion plays a double rôle. It is essential to the mechanism of an effective mechanical contraction and, when unbalanced by potassium, it inhibits the activity of the sodium ion in arousing an inner stimulus. This latter effect of the calcium ion was not clearly shown in the experiments upon the terrapin heart. On a mixture of sodium chloride and calcium chloride in physiological concentrations this heart (like the Maia heart—Hogben) gives rhythmical responses for many hours with excellent mechanical contractions of auricle and ventricle. When the isolated sinus is used and is perfused with a mixture of sodium and calcium chloride until all responses cease, a subsequent perfusion with sodium chloride restores neither the electrical nor the mechanical beat.

Although sodium alone was able to initiate rhythmic activity, the rapidity and certainty of recovery in the sinus upon perfusion with sodium chloride alone, following upon exhaustion of the heart with various solutions, was found to vary directly with the $\frac{C_{Ca}}{C_{Na}}$ ratio in the perfusing liquid with which the heart was exhausted, that is to say, a relative excess of calcium over sodium in the perfusion solution favored subsequent recovery upon a sodium chloride solution. When the perfusing liquid used for exhaustion contained calcium alone, subsequent recovery on sodium was prompt and certain. If the perfusing liquid contained both sodium and calcium in normal proportions, subsequent perfusion with sodium alone never gave any recovery. When the perfusing liquid was dextrose, it may be assumed that the $\frac{C_{Ca}}{C_{Na}}$ ratio was increased owing to the more rapid loss of sodium from the heart, and in these cases recovery by subsequent perfusion with sodium alone occurred only in a certain number of experiments (50 per cent) and was delayed in making its appearance.

With regard to the function of potassium, the experiments demonstrated that it is not essential for the initiation of the beat, as recovery was never produced on potassium after previous exhaustion with dextrose. Potassium, however, appeared to have the function of supplementing sodium

in maintaining the rhythmic activity of the sinus, as shown by the greater duration of the activity on sodium plus potassium than on sodium alone. Potassium also appeared to have the function of controlling the antagonism between sodium and calcium. It will be remembered that in the frog's heart potassium seemed necessary to neutralize the inhibiting effect of calcium upon sodium in the initiation of the inner stimulus. For the terrapin sinus potassium again appeared to be essential as a balancing factor in the presence of sodium and calcium. This was indicated by the fact that perfusion with a potassium-free Ringer's solution so affected the sinus tissue that it was difficult to recover its rhythmicity.

That an electrical response may exist without mechanical contractions has been repeatedly demonstrated throughout this series of experiments. Rhythmic electrical variations were shown to exist in the terrapin sinus after complete cessation of the mechanical contractions when the sinus was perfused with a calcium-free Ringer's solution and in some cases when perfused with sodium alone. The same was true, also, for the auricle and ventricle of the terrapin when perfused with sodium chloride. The reappearance of rhythmic electrical variations in the frog ventricle without any evidence of mechanical contractions, when perfused with sodium following exhaustion on sodium plus calcium, also demonstrated the separability of the electrical and mechanical phenomena. This supports the view of Locke, Mines, and Hogben, and is contrary to that of Einthoven and Arbeiter who believed the two phenomena to be inseparable. Arbeiter claimed that, if the recording instruments were sufficiently sensitive, mechanical contractions in a heart perfused with a calcium-free solution were always seen to survive as long as the electrical variations. In these experiments we have met this objection by using a very sensitive myograph, and a magnification in excess of that employed by Einthoven and Arbeiter. That a lack of sensitiveness of the instrument was not responsible for our results on the calcium-free solutions is indicated by the fact, that, on all solutions containing calcium and on dextrose solutions the electrical and mechanical beats disappeared simultaneously. The experiments indicated that the retention of the electrical response, after the mechanical contractions had disappeared, was a sodium effect, since it was seen upon perfusion with sodium alone as well as with sodium plus potassium.

The chief contribution made by these experiments, we believe, is in stressing again the primary importance of the sodium, potassium, and calcium ions. Whatever may be the part played by organic substances, products of metabolism, the hydrogen ion, etc., these experiments suggest the direct participation of the cations, particularly the sodium ion, in the chemical processes that liberate the inner stimulus, and the necessity of calcium and potassium in supporting and controlling the action of sodium.

That such a form of activity is possible for the inorganic salts seems to be indicated by Warburg's (1930) experiments on cell oxidation, in which he demonstrated the stimulating effect of sodium chloride on cell oxidation and the counteraction of this by calcium.

SUMMARY

1. When the isolated sinus of the terrapin's heart is brought to complete rest by continued perfusion with an isotonic solution of dextrose, rhythmic activity, both electrical and mechanical, although usually small, may be restored for a period by perfusion with solutions containing only sodium chloride.   Solutions containing only calcium or potassium salts, or a combination of the two, have no such effect.   Sodium seems, therefore, to contribute the essential factor in the production of an inner stimulus.

2. The calcium ion appears to be essential to the production of strong, functionally important mechanical contractions.

3. Potassium ions have no specific function in releasing the internal stimulus of the heart.   Their function seems to lie in a regulation of the interaction between the sodium and calcium ions.

4. In the washed frog's heart, there is a distinct antagonism between sodium and calcium in their effect upon rhythmicity.   Solutions containing sodium chloride alone permit or produce rhythmic responses, while the addition of calcium chloride, in physiological concentrations, inhibits all rhythmic activity, so far as can be judged by the electrical and mechanical responses.   In the auricle and ventricle of the terrapin the addition of calcium to the sodium solution does not seem to inhibit rhythmicity although some evidence of this antagonistic effect appears in the terrapin sinus.

5. A rhythmical electrical beat may be exhibited by heart muscle in the complete absence of any mechanical beat.   Such a condition occurs after prolonged perfusion with a solution containing sodium and potassium or sodium alone, i.e., in the absence of calcium.

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THE PHYSIOLOGIC ACTION OF THE VENOM OF THE WATER MOCCASIN (AGKISTRODON PISCIVORUS)

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Study of the venom of the rattlesnake (Crotalus horridus) by Essex and Markowitz (1930) and the venom of the honey bee (Apis mellifera) by Essex, Markowitz and Mann (1930) disclosed a striking similarity in the action of the venom. A study of the venom of the water moccasin was undertaken to determine whether its action differed in any observable particular from these two, since such information would be of much assistance in a study of the specificity of the venoms. Consequently the outstanding physiologic responses following poisoning by rattlesnake and honey bee were investigated with the use of the venom of the water moccasin. This report consists of observations of effects on the blood pressure, the volume of the erythrocytes in vivo and in vitro, intradermal inoculation into human skin, the isolated uterus of the virgin guinea pig and the perfused heart of the rabbit. A 2 per cent solution was prepared from the dried venom of the water moccasin in 50 per cent glycerin and Ringer's solution. The same mixture was used in all of the experiments.

RESULTS. The effect of the venom on the blood pressure of an etherized dog following an intravenous injection of 0.04 cc. of 2 per cent venom of a water moccasin for each kilogram of body weight was typical of the result produced by a comparable dose of crotalin. After a latent period of ten to fifteen seconds the blood pressure fell to about 30 to 40 mm. of mercury. From this point, after ten to fifteen minutes, it gradually returned to a physiologic level which required an hour or more, or if the dose proved lethal, it gradually fell to zero (fig. 1). Its effect on the blood pressure of an etherized rabbit was equally dramatic since an intravenous injection of 0.07 cc. of 2 per cent venom for each kilogram of body weight caused an almost immediate profound fall in blood pressure. If a lethal dose was given the blood pressure remained at a low level but if a sublethal dose was given it returned to a physiologic level in twenty minutes.

That the cause of the vasodepressor action is peripheral, as was found to be the case with crotalin, was shown by plethysmographic studies on the kidney, spleen, and hind limb. A marked decrease in the volume of the

kidney and spleen occurred during the fall in blood pressure but there soon followed a decided increase which usually far exceeded the original volume (fig. 2). In contrast with these observations, the volume of the hind limb was just reversed, since it increased during the fall in blood pressure and decreased soon thereafter. With the assistance of Doctor Herrick, who investigated flow of blood by the technic of Rein, it was shown that these changes in volume are due to distinct alterations in the flow of blood to these organs.
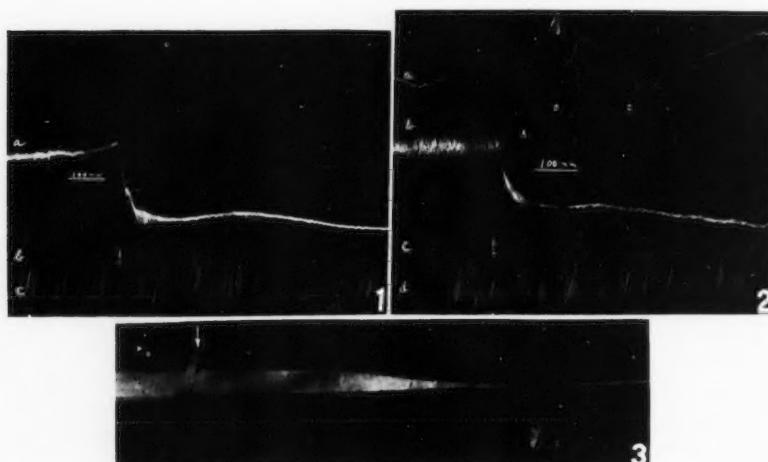
Fig. 1. Lethal effect of an intravenous injection at *1* of 0.04 cc. of 2 per cent venom of a water moccasin for each kilogram of body weight on an etherized dog weighing 11 kgm.; *a*, blood pressure; *b*, 0 mm. of mercury; *c*, time in five second intervals (this is similar in figs. 2 and 3).

Fig. 2. The effect on the splenic volume of 0.04 cc. of 2 per cent venom of a water moccasin given intravenously at *1* to an etherized dog weighing 10.3 kgm.; *A*, piston recorder raised; *B* and *C*, piston recorder lowered; *a*, splenic volume; *b*, blood pressure; *c*, 0 mm. of mercury.

Fig. 3. Tracing made by perfused heart of a rabbit. The arrow indicates the time when 0.05 cc. of 2 per cent of venom of a water moccasin (1 mgm.) was added to 500 cc. of perfusing fluid. The marked decrease in the excursions of the recording lever indicates the extremely toxic action of the venom on the heart.

Many investigators have studied the hemolytic action of the venom of the water moccasin. An intensive study was made by Flexner and Noguchi (1902). However, they confined their attention to observations in vitro, chiefly of defibrinated blood diluted with sodium chloride solution. They were concerned with the complete hemolysis of the blood whereas my observations deal only with the precursor of hemolysis, namely, the swelling of the erythrocytes.

A comparison of samples of 15 cc. of blood drawn into heparin before and after the injection of the venom showed marked increase in the volume of the erythrocytes in the tubes taken from three to six minutes after injection.   All tubes were centrifuged for twenty minutes at 1,500 revolutions a minute.   As in previous studies it was found by observations made in vitro that the changes in volume occurring in vivo were due to an increase in the volume of the erythrocytes.

In dog 1 the control volume of erythrocytes was 5.8 cc. three minutes following the intravenous injection of 0.8 mgm. of venom of the water moccasin for each kilogram.   The volume of erythrocytes was 8.3 cc., an increase of 43 per cent; in dog 2 the control volume of erythrocytes was 8.1; four minutes following intravenous injection of 0.8 mgm. of venom of the water moccasin the volume of erythrocytes was 11 cc., an increase of 36 per cent, and in dog 3 the control volume of erythrocytes was 7.8; six minutes following intravenous injection of 0.8 mgm. of venom of the water moccasin the volume of erythrocytes was 12.5, an increase of 60 per cent.

The effect of the venom of the water moccasin on the guinea pig is also identical with that of the venom of the rattlesnake.   Intravenous injections produced occlusive bronchospasm and death about ten minutes after a lethal dose had been given.   The symptoms briefly are roughening of the coat, wheezing, and gasping.   At necropsy the lungs appear markedly distended.

The uterus of the virgin guinea pig when perfused by the method of Dale responded to 1 mgm. of venom of the water moccasin in 40 cc. perfusing fluid by maximal contraction.   Three or four changes of fluid were necessary to produce relaxation and rhythmic contractions, after which a second dose elicited a similar response.

The isolated heart of the rabbit, perfused according to the method of Locke and Rosenheim modified to permit tracings, was incapacitated within ten to fifteen minutes after the addition of 1 to 2 mgm. of venom of the water moccasin to 500 cc. of Ringer-Locke's solution (fig. 3).

In these experiments attempts were not made to determine if a narrow margin of difference exists in the toxicity of the venom of the rattlesnake and that of the water moccasin.   It has seemed sufficient for our purposes to use what might be considered comparable doses of the two venoms.   It should be emphasized that dilutions of venom of the water moccasin were made from dried material.

SUMMARY AND CONCLUSIONS

Venom of the water moccasin when given intravenously to an etherized dog or rabbit causes a profound fall in blood pressure.   That the action of the venom is peripheral has been shown by plethysmographic studies on the spleen, kidney and hind limb.   A decided increase in the volume

of the erythrocytes occurs following the addition of the venom in vivo or vitro.   When it is injected intradermally into the human skin, reddening, whealing, and an arteriolar flare result.   The perfused uterus of the virgin guinea pig responds by maximal contraction and the isolated heart of the rabbit is quickly incapacitated by small doses added to the perfusing fluid.   Intravenous injections into guinea pigs produce occlusive bronchospasm.

The evidence obtained indicates that there is not a distinguishable difference in the physiologic action of the venom of the water moccasin and that of the rattlesnake as judged by the tests employed.   It should not, however, be inferred that the venoms are the same in their fundamental composition.

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SPECIFICITY OF IMMUNITY TO VENOM OF A RATTLESNAKE AS INDICATED BY INJECTIONS OF VENOM OF THE WATER MOCCASIN AND HONEY BEE

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Studies on the venom of the rattlesnake, the honey bee, and the water moccasin have indicated close similarity in physiologic action. In spite of the well known highly specific nature of proteins it seemed desirable to observe whether active immunity to one of these venoms afforded any protection against the action of the others, as measured by criteria utilized in previous investigations. For this study dogs immunized against crotalin were given injections of the venom of the water moccasin (Agkistrodon piscivorus) and honey bee (Apis mellifera). Noguchi (1906) concluded from an investigation of the specificity of snake antiserums that 2.5 cc. of rattlesnake antiserum protected against 1 minimum lethal dose of venom of water moccasin in guinea pigs. Rogers (1904) in a series of experiments reported protection against several snake venoms when Calmette's antiserum was used in large doses. It may be added, however, that the doses were so large as to raise considerable doubt as to their therapeutic value.

Since it has been shown in previous work that the effect on the blood pressure and the erythrocytes of the dog may be taken as reliable criteria of the relative immunity possessed by dogs immunized against crotalin, these test objects have been utilized in the present study. So far as I am aware, animals actively immunized to a given venom have never been utilized in investigations of specificity. Previous studies have been made chiefly on guinea pigs with mixtures of venom and antiserum, and the minimal lethal dose has been determined. The solutions of venom for these experiments were prepared from dried material. The rattlesnake venom used was obtained from Crotalus horridus.

For the experiments here reported, dogs highly immune to crotalin were utilized and the effect on the erythrocytes and blood pressure was observed after the injection of the proper dose of venom of the water moccasin and honey bee. In each instance, as a control, the same animals were given the same or a larger dose of crotalin after they had recovered. In each experiment the blood pressure was taken from the femoral or brachial artery.

RESULTS. An intravenous injection of 0.04 cc. of 2 per cent venom of the water moccasin for each kilogram of body weight was given during a period of one minute to a dog weighing 22 kgm. with an antihemolytic titer of 152. Before the injection was completed the blood pressure dropped precipitately, and within a minute had fallen to about 50 mm. of mercury where it remained for about six minutes before gradually rising to a physiologic
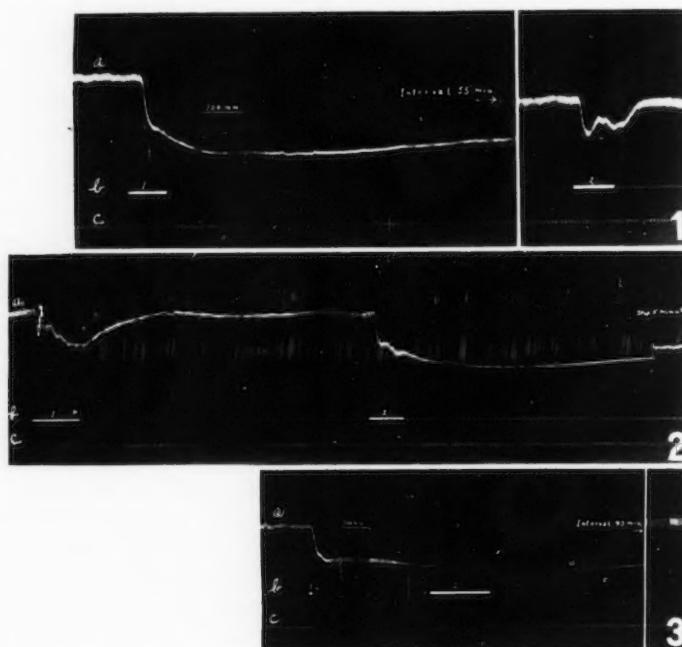

Fig. 1. *1*, the effect on the blood pressure of an intravenous injection of 0.04 cc. of 2 per cent venom of water moccasin for each kilogram of body weight into a dog that weighed 22 kgm. with considerable immunity to rattlesnake venom; *2*, the effect of the same dose of the venom of a rattlesnake. For this and succeeding figures, *a* equals blood pressure; *b*, 0. mm. of mercury, and *c*, time in intervals of five seconds.

Fig. 2. *1*, the reaction of the blood pressure to an initial injection of 0.04 cc. of 2 per cent venom of rattlesnake for each kilogram of body weight when given to a dog that weighed 23.4 kgm. possessing considerable immunity to rattlesnake venom; *2*, the effect of half the dose of venom of water moccasin given intravenously about eight minutes later.

Fig. 3. *1*, the effect on the blood pressure of a normal dog weighing 2.8 kgm. of an intravenous injection of 0.04 cc. of 2 per cent venom of water moccasin for each kilogram of body weight; *2*, 10 cc. of plasma from a dog highly immune to venom of rattle snake was given intravenously without increasing the period of recovery over normal dogs to which such injections were given.

level, which required a little more than an hour.   When the blood pressure
had reached a level of about 110 mm. of mercury the same dose of crotalin
was given intravenously with a very different result.   There followed a
marked depressor response of about 30 mm. of mercury, but complete
recovery had occurred in less than a minute following completion of the
injection (fig. 1).

In the next experiment, an initial injection of 0.04 cc. of 2 per cent crota-
lin was given intravenously over a period of one minute to a dog with an
antihemolytic titer for crotalin of 160.   The blood pressure fell in this case
about 50 mm. of mercury, but had fully recovered in less than three min-
utes.   After an interval of about six minutes, during which the blood
pressure was maintained at the original level, an intravenous injection
of 0.02 cc. of venom of the water moccasin (half the dose of crotalin) was
given, with a markedly different result.   The blood pressure dropped to
about 80 mm. of mercury and remained there for about five minutes.   It
had not attained the original level (160 mm. of mercury) twenty minutes
after the injection (fig. 2).

If immunity to crotalin affords any protection against the venom of the
water moccasin it should be possible readily to overcome the fall in blood
pressure produced by the latter when given to a normal dog by intravenous
injections of plasma from a highly immune dog.   In the next experiment
a small dog weighing 2.8 kgm. was used.   An intravenous injection of
0.04 cc. of 2 per cent venom of the water moccasin was given with the
typical result.   The blood pressure fell from about 100 mm. of mercury
to about 40.   Three minutes after this injection 10 cc. of plasma from a
dog highly immune to crotalin was given intravenously over a period of
two minutes.   The time necessary for the blood pressure to reach a
physiologic level was not less than that for normal dogs to which antiserum
was not given following the injection of venom of the water moccasin
(fig. 3).

One of the outstanding characteristics of snake venom is its hemolytic
action.   In previous reports it has been pointed out that as a precursor to
hemolysis a decided increase in the volume of the erythrocytes occurs.
Large doses of crotalin are necessary to produce any change in the volume
of the erythrocytes of immune dogs.   In each of the experiments just
described 15 cc. of blood were drawn into tubes containing heparin before
and after the injections of venom.   In the second experiment, crotalin was
injected into a highly immune dog, but increase in the volume of the eryth-
rocytes did not occur following this injection.   However, there was a
marked increase in the volume of the erythrocytes following an injection
of half as much venom of the water moccasin.   The cell volume of the
three tubes read as follows: 1, control, 10 cc.; 2, following crotalin, 10
cc., and 3, following venom of the water moccasin, 14 cc.   In the first

experiment described the initial injection was venom of the water moccasin. Following this injection a decided increase in the volume of the erythrocytes occurred. The cell volume before and after the injection was 7.5 cc. and 11 cc., respectively. A similar result was obtained in the third experiment.

By a comparable series of experiments it has been shown that dogs immunized to crotalin do not possess any appreciable protection against the venom of the honey bee. The physiologic reactions of such dogs are so nearly identical with those of normal dogs, described in a former paper, that further comment is unnecessary.

COMMENT. It is not surprising that immunity to crotalin does not afford any protection against the venom of the honey bee. Considering the fact that the rattlesnake and the bee belong to such widely divergent branches of the phylogenetic tree and the origin of their venoms is so dissimilar, the results obtained were to be expected. This, however, does not hold true for the venom of the water moccasin which is a close relative of the rattlesnake, both belonging to the group known as the pit vipers. Furthermore, the literature contains statements that a low degree of protection against the venom of the water moccasin is afforded by rattlesnake antiserum. Consequently, the results obtained in the experiments reported here occasioned some surprise since there was no evidence, so far as I could judge, that rattlesnake antiserum protects even slightly against the venom of the water moccasin.

SUMMARY

Dogs actively immunized to rattlesnake venom are not afforded appreciable protection against the venom of the water moccasin or honey bee, as judged by the effect on the blood pressure and volume of erythrocytes following intravenous injections into dogs whose blood contains a relatively large amount of crotalin antibody. Plasma from a dog possessing a high degree of immunity to crotalin failed to speed up recovery of the blood pressure after it had been lowered by the venom of the water moccasin.

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STUDIES IN THE PHYSIOLOGY OF VITAMINS

XVII. The Effect of Thyroid Administration upon the Anorexia Characteristic of Lack of Undifferentiated Vitamin B

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A relationship between food intake and the requirement for undifferentiated vitamin B, or so-called vitamin B complex, has been shown by various investigators (Voegtlin and Lake, 1918; Karr, 1920; Cowgill, 1921). Osborne and Mendel (1922) noticed that the amount of this dietary factor required for growth of the white rat over a given period is in general related to the size of the animal as expressed by weight. Further study along this line, summarized by Cowgill and Klotz (1927), has indicated that the vitamin B requirement in different species of animals may be approximated fairly accurately by means of the expression

$$\frac{\text{Vitamin}}{\text{Weight}^{5/3}} = K,$$

which was interpreted to mean

$$\frac{\text{Vitamin}}{\text{Weight} \times \text{Calories}} = K.$$

A similar relationship, namely,

$$\frac{\text{Vitamin B}}{\text{Calories}} = K,$$

was pointed out by Plimmer, Rosedale and Raymond (1927). It will be noticed that *calories* is one of the factors in each of the last two formulae cited above. If these generalizations are correct, it ought to be possible to show that, with the body weight maintained at essentially the same level, the vitamin requirement of a given animal is significantly greater when the calorie factor is increased. This has already been shown to be true when the voluntary food intake is increased through vigorous exercise (Cowgill, Rosenberg and Rogoff, 1931b). Another way in which the metabolism factor may be increased experimentally is by inducing a condition of

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hyperthyroidism. The present paper is a report of observations of the dog's requirement for undifferentiated vitamin[1] during a control period and when in a state of experimentally induced hyperthyroidism.

EXPERIMENTAL PART. *Methods.* Studies made in these laboratories (Cowgill, 1923) have shown that most dogs require about three weeks subsistence on a vitamin B-deficient diet of the type *casein III* described elsewhere (Cowgill, 1928) in order to develop the anorexia characteristic of lack of this dietary factor. This period is an average one, however. It is necessary, therefore, to determine the period for each experimental animal. The minimum amount of the artificial food mixture containing vitamin B, that will serve to maintain the animal's body weight under the conditions of cage life, is determined. The dog is then "saturated with vitamin B" by several large successive daily doses of some good source of this important food factor. The basal diet alone, without vitamin supplement, is then fed and the number of days required for appearance of the characteristic anorexia is again determined.

In the present study hyperthyroidism was induced by administration of 5 grams of desiccated thyroid (Armour's) daily over periods indicated for the respective dogs in table 1.

Results. The observations reported in this paper were made on four dogs. Animals 1 and 2 had previously maintained their urge to eat a diet of commercial dog biscuit satisfactorily over a period of at least three months. When they were given 5 grams of desiccated thyroid daily, they exhibited a marked loss of appetite for the ration after two weeks, and, as a consequence, lost considerable weight. During the next three weeks occasional administrations of undifferentiated vitamin B were followed in every case by a restoration of the urge to eat, as evidenced by the daily voluntary consumption of larger amounts of food. These observations were so striking that it was deemed advisable to feed an artificial ration (diet *casein III*, Cowgill, 1928) of known composition, and to measure the food intake as accurately as possible. For 18 days the animals were given 10 grams per day of a vitamin B concentrate[2] as part of the experimental routine, as a result of which they proceeded to regain their initial weight. The degree of hyperthyroidism induced is indicated by the fact that on the

[1] The recent studies of Cowgill, Rosenberg and Rogoff (1931a), Burack and Cowgill (1931), and Sherman and Sandels (1931), indicate that the antineuritic component of the so-called vitamin B complex is the chief etiologic agent with respect to the development of the anorexia characteristic of "vitamin B deficiency;" the G or B_2 factor does not play a rôle. The observations reported in this paper, therefore, in all probability pertain to the antineuritic B_1 substance. The preparation used in these experiments as a source of vitamin B supplied the complex. Therefore, in this paper the term vitamin B is used to mean the undifferentiated B complex.

[2] Yeast vitamine powder (Harris), kindly supplied by the Harris Laboratories, Tuckahoe, New York.

56th day of thyroid administration the animals were voluntarily eating an amount of the artificial ration containing approximately 1200 calories, an intake about twice that characterising dogs of similar size but not receiving thyroid (Cowgill, 1928). The administration of vitamin B was then discontinued, and, as a result, anorexia appeared in 20 and 17 days with dogs 1 and 2 respectively. During their respective periods animal 1 consumed an average of 1166 calories, and dog 2 an average of 1231 calories per day. In both cases, administration of the missing vitamin was followed by a return of the urge to eat. The thyroid administration was then discontinued and the animals were allowed a period

TABLE 1

The caloric intake as affected by the administration of desiccated thyroid

PERIOD OF VOLUNTARY FOOD INTAKE		DOG 1	DOG 2	DOG 3	DOG 4	AVERAGE FOR THE GROUP
Control period, without thyroid	Days in the period..............	34	39	23	31	32
	Calories ingested per day..........	600	720	656	635	650
	Total Calories ingested for the period...........	20,400	28,080	15,088	19,685	20,813
Experimental, hyperthyroidism	Days in the period..............	20	17	12	20	17
	Calories ingested per day..........	1,166	1,231	1,011	1,213	1,155
	Total Calories ingested for the period...........	23,320	20,927	12,132	24,260	20,160
Average total Calories ingested......		21,860	24,503	13,610	21,872	20,486 (group) 20,461 (individuals)

of six months during which to recover from the effects of the thyroid. The vitamin B requirement was again determined for these dogs using the plan described under *methods* above. Dog 1 exhibited anorexia after 34 days during which it ate an amount of the ration approximating 600 calories per day; and dog 2 lost the desire to eat after 39 days during which period it ate on an average 720 calories per day.

In the experiments with dogs 3 and 4 the periods required for the characteristic anorexia to develop under "normal" conditions were determined first, and then the effect of thyroid administration was studied. The data are summarized in table 1.

It will be noticed in table 1 that the caloric intakes of the *individual dogs* until anorexia appeared, as well as the *animals considered as a group* were fairly constant either with or without the administration of thyroid. The data in table 2 show that the loss of body weight so characteristic of hyperthyroidism is readily corrected by administration of undifferentiated vitamin B. Obviously the loss in weight is due chiefly, if not solely, to an insufficient food intake.

DISCUSSION. From table 1 it is evident that the requirement of the organism for undifferentiated vitamin B bears a close relationship to the caloric intake. Suggestions have been made that the vitamin requirement is closely related to the total metabolism, of which the caloric intake may be taken as a rough measure (Osborne and Mendel, 1922; Cowgill and

TABLE 2

The effect of administration of vitamin B on the body weight in experimental hyperthyroidism

	BODY WEIGHT (KILOS)			
	Dog 1	Dog 2	Dog 3	Dog 4
Initial..	11.5	12.5	14.4	10.5
After thyroid administration for number of days indicated............................	10.6 14 days	11.1 14 days	13.2 17 days	9.5 24 days
After 21 days of occasional administrations of vitamin B..............................	9.9	8.5		
After daily administrations of vitamin B for number of days indicated................	11.5 18 days	10.6 18 days	13.9 5 days	10.4 9 days

Klotz, 1927). In summing up the findings of researches carried out on different species of animals, Cowgill and Klotz stated the relationship as follows: "the vitamin B requirement appears to be proportional to the mass of tissue, i.e., body weight, and the metabolism of that mass, namely, calories;" or, "the vitamin requirement per unit of tissue mass is proportional to the metabolism of that mass." The findings of the present study are in accord with this view. Inasmuch as the dogs used in this investigation were of about the same weight, and therefore possessed about the same mass of tissue, their vitamin requirements, in the light of the above dicta, would be proportional to their respective total metabolisms, of which the food intake would be a rough measure.

There are various reasons why the caloric intake can be regarded only as a

first approximation to the true metabolism.   Evidence is at hand showing
that in hyperthyroidism changes occur in the proportions of the various
foodstuffs that are oxidized.   Kommerell (1929) observed that the increase
in metabolism due to thyroid administration in starved animals was
accounted for by a 31 per cent increase in oxidation of protein and 69 per
cent increase in fat catabolism.   Sanger and Hun (1922) also noted an
acidosis in hyperthyroidism when the carbohydrate stores were depleted.
Determinations carried out on the urines of dogs 1 and 2 during the first
32 days of thyroid administration showed that small amounts of acetone
were being eliminated daily.   The loss of energy represented by the
acetone bodies excreted through the lungs and kidneys would be another
source of error, if the food intake was taken as a measure of the total
metabolism, particularly during hyperthyroidism.   In the light of these
considerations, therefore, the agreement of the data for voluntarily ingested
calories in the two periods, namely, with and without thyroid administra-
tion, may be regarded as good.   Evidently, then, the tissue stores of
vitamin B were drawn upon during the periods when the animals main-
tained their normal urge to eat, and this supply was consumed in the
metabolism of a fairly definite quantity of foodstuffs; the amount oxidized
during the period of hyperthyroidism was about the same as that for the
control period.   This finding emphasizes once more the relation of vitamin
B to the metabolism of one or more of the foodstuffs, a conclusion which
numerous investigators have reached (Funk, 1914; Randoin and Simonnet,
1924; Evans and Lepkovsky, 1928).

This increase in the vitamin B requirement associated with a rise in the
metabolic rate may explain some of the observations reported in the litera-
ture.   Kunde (1927), in her study of hyperthyroidism in dogs, noticed
that some of her animals lost considerable weight in contrast to other dogs
in which maintenance of weight occurred.   In view of the data reported in
this paper it seems reasonable to assume that vitamin B deficiency was a
complicating factor in Kunde's experiments, and that the animals which
lost weight needed more of this dietary factor, presumably to maintain the
urge to eat.   Steenbock, Sell and Nelson (1923) and others have shown
that when rats have access to their intestinal excreta, they require a smaller
amount of vitamin B in the food than is the case when coprophagy is
prevented.   Similar observations on the dog have been made in this
laboratory.   It is possible that those of Kunde's animals that maintained
their weights, were coprophagists.   Abelin, Knuchel and Spichtin (1930)
observed that depletion of glycogen stores of liver and muscle in hyper-
thyroidism is prevented when the animals are fed diets rich in vitamins; no
data were published by these investigators concerning the amounts of food
consumed in their experiments, but it is significant that the animals which
subsisted on the rations containing large amounts of vitamins showed a

weight loss only half as great as those fed on diets low in vitamin content. Such a result might be expected in view of the findings reported in this paper.

The writers believe that the results of this investigation are of clinical significance. The marked loss of body weight characteristic of hyperthyroidism is well known; in the light of these experiments it is quite possible that vitamin B deficiency is an etiologic factor here. Administration of large amounts of vitamin B to cases of hyperthyroidism is certainly worthy of clinical trial. If one were permitted to generalize, it might be said that vitamin B therapy is indicated in those conditions characterized by an increase in the metabolic rate.

SUMMARY—CONCLUSIONS

The time required for development of the anorexia characteristic of vitamin B deficiency, and the amount of food ingested during the period of voluntary food intake were determined in four dogs under "normal" or basal conditions and during experimental hyperthyroidism. It was found that during hyperthyroidism 1, anorexia appeared in from one-half to two-thirds of the time required during the control period; and 2, the quantity of food ingested voluntarily per day was correspondingly increased. The total caloric intakes for the two experimental periods were approximately the same. This suggests that a definite relationship exists between a given amount of vitamin B and the catabolism of a definite quantity of foodstuffs. The four animals successfully maintained their weights by voluntary ingestion of food only when receiving sufficient amounts of vitamin B.

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OBSERVATIONS ON THE FLOW OF BLOOD OF THE KIDNEY[1]

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Studies on the flow of blood through mammalian organs have occupied the attention of many workers and consequently considerable data on the subject have accumulated. All of the methods used in the past have been open to criticism, and it is improbable that a method of measuring flow of blood will ever be devised against which objections may not be raised. However, it is not unreasonable to expect that improved methods will be presented occasionally by the use of which a closer approximation to the normal flow of blood of an organ will be made possible. The data presented in this report were obtained by the use of a new method recently described by Rein (1928) and modified slightly by Herrick and Baldes (1931). This method is free from certain of the objections raised against the apparatus and technic employed by previous workers. Rein's method, known as the "Thermo-Stromuhr," permits the continuous measurement of the average absolute quantity of blood flowing in closed vessels with a minimum of manipulation, the apparatus being applied to the vessel in situ. It is, undoubtedly, the most physiologic of all quantitative methods developed to the present time. We are reporting here data collected on: 1, the flow of blood of the kidney studied simultaneously with plethysmographic changes; 2, the effect of the removal of one kidney on the flow of blood of the other, and 3, the flow of blood of the transplanted kidney.

On reviewing the literature there seems to be no instance in which plethysmographic observations have been made simultaneously with actual quantitative observations of the flow of blood. Since the plethysmographic method is employed so generally for studying the vasodilatation and vasoconstriction in an organ, it was considered important to compare the variations in the volume of an organ simultaneously with the corresponding changes in the flow of blood. Perhaps these observations have not been made previously because a convenient method for measuring the flow of blood during the recording of changes in volume indicated by the

plethysmograph was not available. The Thermo-Stromuhr permits this with considerable ease.

After applying the plethysmograph to the kidney and the Thermo-Stromuhr to the renal artery or renal vein, changes in volume were produced by various drugs and diuretics. The drugs used were epinephrine, ephedrine, histamine, snake venom and nicotine. The diuretics were caffeine, saline, glucose and sodium sulphate. In every instance an increase in the volume of the kidney was accompanied by an increase in the flow of blood, and a decrease in the volume of the kidney was accompanied by a decrease in the flow of blood. Because air, instead of a liquid, was the medium of communication between the plethysmograph and the piston recorder we did not attempt to compare the two methods quantitatively. The primary purpose in performing this series of observations was to see whether the increases or decreases of volume recorded by the plethysmograph corresponded to increases or decreases in flow of blood as indicated by the Thermo-Stromuhr. Representative results are shown in figures 1 and 2. Figure 1 illustrates the results obtained when 0.1 cc. of 1:1000 epinephrine was injected intravenously in a dog weighing 15.5 kgm. (anesthesia by sodium iso-amytalethyl barbiturate). In this instance measurements of flow of blood were made on the renal artery. Figure 2 shows the variations produced by injecting 15 mgm. of ephedrine in a dog weighing 16.8 kgm., using the same anesthesia. In this experiment the flow of blood in the renal vein was measured.

Each diuretic which was employed caused simultaneous increases both in renal volume and in flow of blood during the period of injection. A 2.5 per cent solution of sodium sulphate produced the greatest increase; the results during its injection are shown in figure 3. Two hundred cubic centimeters were injected intravenously in a dog weighing 20.2 kgm. Great care was taken to maintain blood pressure as constant as possible during the injection. The dog had been given 500 cc. of water by stomach tube three hours before the experiment was begun. Observations were continued during the subsequent diuresis. It was found that the flow of blood did not increase during diuresis. In many instances these observations extended for two or more hours after the diuretic was injected. During this time the influence of the blood pressure on the flow of blood to the kidney was observed. It is well known that the variations in the flow of blood parallel the corresponding variations in general systemic pressure provided drugs that cause local vasoconstriction or vasodilatation have not been given.

The foregoing data do not reveal anything new in regard to the effect of the given substances on the flow of blood to the kidney. These particular effects have been established previously by other methods. However, there seems to be some doubt as to whether the variations in the
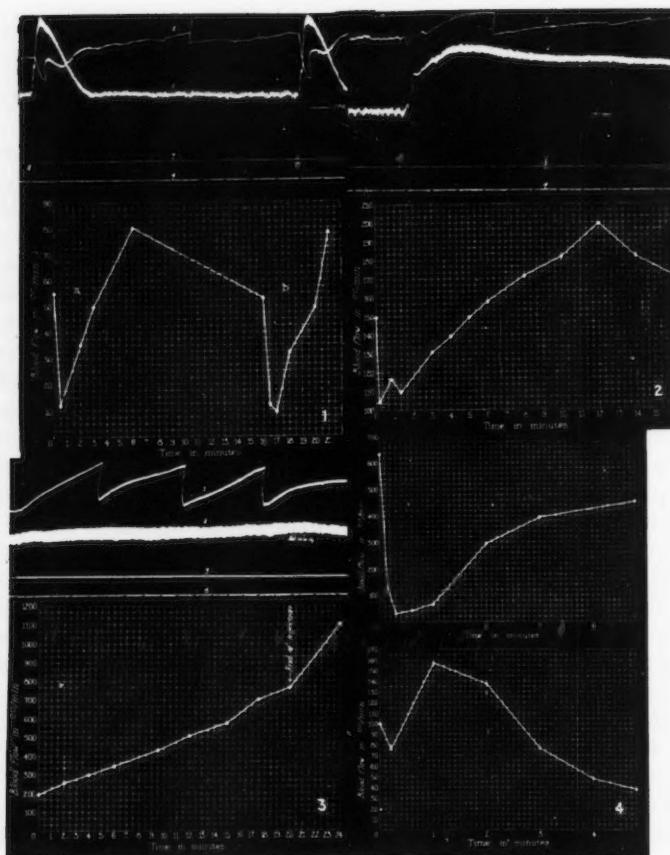
Fig. 1. The effect of 0.1 cc. of 1:1000 epinephrine. Two injections were made at A and B respectively. The accompanying chart indicates, at a and b, the changes in the flow of blood in the renal artery following the injections of epinephrine at A and B. Weight of dog, 15.5 kgm. (Figs. 1, 2 and 3 record the effect of the intravenous injection into dogs of certain drugs on renal volume, blood pressure and flow of blood. The changes in the flow of blood are shown in the chart accompanying each kymogram. In each of the figures: 1 = kidney volume; 2 = blood pressure; 3 = 0 mm. of mercury, and 4 = time in intervals of 5 seconds.)

Fig. 2. The effect of 15 mgm. of ephedrine. The accompanying chart indicates the changes in the flow of blood in the renal vein following the injection at A. The first point on the chart indicates the flow of blood at the beginning of the injection. Weight of dog, 16.8 kgm.

Fig. 3. The effect of 200 cc. of 2.5 per cent solution of sodium sulphate. The accompanying chart indicates the changes in the flow of blood in the renal artery during the period of injection. The first point on the chart indicates the flow of blood at the beginning of the injection. Weight of dog, 20.2 kgm.

Fig. 4. The upper chart shows the changes in the flow of blood in the carotid artery anastomosed to the renal artery following the intravenous injection of 0.2 cc. of 1:1000 epinephrine. The lower chart shows the changes in flow of blood in the intact carotid following the same injection. Weight of dog, 21 kgm.

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volume of the kidney, as indicated by the plethysmograph, may be inter-
preted as due to corresponding variations in the flow of blood.   One might
easily evoke reasons for an increase in the volume while the flow remains
constant.   Although we do not propose to argue against such possibilities,
we can say that in the foregoing instances the changes in volume were
definitely accompanied by corresponding changes in the flow of blood.
Furthermore, this technic may serve to settle any such controversies
which might arise in the future.   Whether the change in volume is due to
the single factor of flow of blood remains unsolved.

THE EFFECT OF REMOVAL OF ONE KIDNEY.   In the intact animal the
function of renal excretion is doubtless equally divided between the two
kidneys.   It was considered of interest to determine whether after removal
of one kidney the remaining kidney quickly assumed an additional burden.
This might reasonably be considered to have happened if a decided increase
in the flow of blood occurred after unilateral nephrectomy.

Two dogs were used for this study.   The dogs were kept under ether
anesthesia and the flow of blood in the renal artery was observed.   The
flow of blood of one kidney was observed for a considerable time before
the other was removed from the circulation; this removal was accomplished
by ligating tightly both the renal vein and the renal artery and excising
the organ.   Following the removal, observations on the flow of blood of
the first kidney were continued for about three hours.   At the end of this
time, because of the prolonged anesthesia, the dog could not be considered
normal.   The results of these two experiments were as follows:

In the first experiment, on a dog weighing 18.2 kgm., the average flow
of blood each minute in the renal artery of a kidney before removal of the
other was 725 cc.; the average flow each minute in the renal artery of the
same kidney after the removal of the other kidney was 665 cc.   In the
second dog, weighing 19.8 kgm. the average flow of blood each minute in
the renal artery of a kidney before removal of the other was 177 cc.; the
average flow each minute in the renal artery of the same kidney after the
removal of the other kidney was 181 cc.   The greater flow of blood in the
first experiment is due to the fact that the observations were made after
the renal nerves had been sectioned.   It might be stated that the flow
before sectioning was 142 cc. a minute.   The act of sectioning caused
immediate constriction resulting in a flow of 47 cc. a minute.   This in turn
was followed by a marked increase in flow.   The renal nerves were un-
injured in the second experiment.   These results show that the removal
of one kidney had no effect on the flow of blood of the other kidney during
the period of observation.   No doubt, this period of observation is too
brief to detect the ultimate effect.

THE FLOW OF BLOOD OF THE TRANSPLANTED KIDNEY.[2]   It was shown to

[2] These transplantations were performed by Dr. P. P. T. Wu.

our satisfaction that sectioning of the nerves of the kidney resulted in an increased flow of blood to the kidney.   The question naturally arises as to whether the resulting vasodilatation is of long duration.   A transplanted kidney is unquestionably completely denervated, and a study of the flow of blood to such a kidney would indicate whether the vessels recover their tone in a relatively short time or whether the vasodilatation persists. Furthermore, the behavior of such a transplanted kidney in the presence of certain drugs should be instructive as compared to that of the normal organ.

Two dogs under ether anesthesia were used in these experiments.   The kidney was transplanted in the neck; the renal artery was anastomosed to the carotid artery and the renal vein to the jugular vein.   After sufficient time had elapsed for recovery from the operation, about three days, measurements of the flow of blood were made on the carotid artery which was anastomosed to the renal artery.   In each experiment the transplanted kidney seemed to be functioning normally, secreting about 0.5 cc. of urine each minute.   After the flow of blood in the carotid artery had been well established glucose was injected intravenously.   When the effect of this had worn off sodium sulphate was injected.   Finally, 0.2 cc. of epinephrine was injected intravenously.   Having completed the desired observations on the carotid artery which was anastomosed to the renal artery, the Thermo-Stromuhr was transferred to the normal carotid artery. Observations were made during and after the injection of epinephrine. The tabulation shows the results obtained from one of the dogs (weight 21 kgm.).

BLOOD VESSEL EXAMINED	SUBSTANCE INJECTED	FLOW OF BLOOD AT BEGINNING OF INJECTION	FLOW OF BLOOD IMMEDIATELY FOLLOWING INJECTION	FLOW OF BLOOD A FEW MINUTES LATER	FLOW OF BLOOD SEVERAL MINUTES LATER
Carotid artery anastomosed to renal artery	Glucose, 300 cc. of 10 per cent solution	144	144	205	163
Carotid artery anastomosed to renal artery	Na$_2$SO$_4$, 300 cc. of 2 per cent solution	228	228	468	637
Carotid artery anastomosed to renal artery	Epinephrine, 0.2 cc.	637	134	32	453
Normal carotid artery	Epinephrine, 0.2 cc.	76.7	158	77	37.7

Figure 4 shows the variations in the flow of blood in both the normal carotid artery and the carotid artery anastomosed to the renal artery when epinephrine was injected intravenously.   The second dog weighed only 8.5 kgm.   The carotid artery anastomosed to the renal artery had a flow of

blood of 87 cc. a minute. It responded similarly to the diuretics and to epinephrine.

If one were to calculate the flow of blood of the kidney according to the figures stated in Cushny (p. 43) one would find that 144 cc. a minute is slightly low for a dog weighing 21 kgm. and that 87 cc. a minute is slightly high for a dog weighing 8.5 kgm. However, these figures do not differ markedly from those for the normal intact kidney. The transplanted kidney responds to the diuretics, glucose and sodium sulphate, similarly to the normal kidney. Epinephrine also produced the same vasoconstricting effect. It is interesting to compare the action of epinephrine on the flow of blood of the normal carotid artery with that of the carotid artery anastomosed in the renal artery. The normal carotid artery responds in its characteristic way by indicating an increase in flow whereas the other shows a marked decrease in flow.

SUMMARY

Measurements of the flow of blood, using the method of the Thermo-Stromuhr of Rein, were made on the renal artery or renal vein while a plethysmograph was recording changes in volume of the same kidney. These changes were brought about by various drugs and diuretics. In every instance it was found that an increase in volume was accompanied by an increase in the flow of blood and that a decrease in volume was accompanied by a decrease in the flow of blood.

The removal of one kidney from the circulation seems to have no effect on the flow of blood to the other kidney throughout a period of about three hours.

The flow of blood to the transplanted kidney seems to be about that of the intact kidney. Glucose, sodium sulphate and epinephrine have the same effect on the flow of blood to the transplanted kidney as they have on the normal kidney.

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THE ACCUMULATION OF LACTIC ACID IN EXCISED BRAIN, KIDNEY, MUSCLE AND TESTICLE

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The structural, functional and chemical differences of the various organs suggest the probability of characteristic differences in lactic acid metabolism. Although numerous studies on the lactic acid content of individual organs confirm this supposition, conclusions deduced from data acquired under different experimental conditions might lead to an incorrect representation of the relative lactic acid metabolism of the various tissues of a given animal. The present investigation was undertaken to obtain with identical experimental procedure, data on the normal lactic acid content of the brain, kidney, muscle and testicle of the dog and to determine the rate at which lactic acid is formed in the tissues under asphyxial conditions.

METHOD. Small dogs weighing approximately four kilograms were used in all the experiments. The animal was decapitated by means of a T-shaped guillotine described by McGinty and Gesell (1925). The brain was removed quickly and the anterior portion of one of the cerebral hemispheres (approximately 8 to 10 grams of brain tissue) frozen in liquid air. The tissue was dropped into the liquid air within 10 to 20 seconds after decapitation. The corresponding portion of the other half of the brain was incubated in an oven at 38°C. and at specified intervals was also frozen. Both samples were later analyzed for lactic acid. The difference between the lactic acid content of the first and second sample per 100 grams of tissue was taken as the amount of lactic acid formed during the period of incubation. The same procedure was followed with the other organs with the exception that the entire organ (kidney, testicle, muscle) was frozen. Time intervals between decapitation and freezing of the tissues were recorded with a stop watch. The brain, kidney and testicle were easily removed within a few seconds after decapitating the animal but rapid removal of the muscle presented some difficulties. In an early series of experiments the sartorius muscle was quickly dissected out but when the lactic acid values were plotted the points were scattered. Thinking that these variations were probably due to unavoidable stimulation and injury in removing the sartorius muscle, another series of experi-

ments was undertaken on the vastus lateralis. This thick, round, large muscle can be removed with but little stretching and the error thereby introduced would be minimized. From the values obtained with this muscle the curves in figures 3 and 3a were constructed.

The tissues, when completely frozen, were pulverized in a cold room with a specially designed chromium-plated brass mortar and pestle. With this instrument the tissue could be ground into a fine powder within a few minutes. Liquid air, poured frequently on the tissue while it was being pulverized, kept it thoroughly frozen. The pulverized tissue was then weighed and mixed with sand in a mortar. Proteins were precipitated with ten per cent sodium tungstate and $\frac{2}{3}$ N sulphuric acid. The tissue was then ground into a thin brei and the contents of the mortar poured into a beaker to which was added the several rinsings of the mortar. Sugar was removed with copper sulphate and calcium hydroxide (Van Slyke, 1917). Determinations of lactic acid were made on aliquot portions according to the Friedemann, Cotonio and Shaffer procedure (1927).

RESULTS AND DISCUSSION. One set of curves, figures 1–4, is plotted from difference values, the other set from absolute values. It will be noted that more data are included in the latter. In an earlier series of experiments all the organs were dropped simultaneously into liquid air, eighty seconds on the average being required for removal and freezing of the tissues. Since the formation of lactic acid is extremely rapid during the first five minutes accurate difference values between two organs can not be obtained unless one organ is frozen within a few seconds after decapitation. For this reason the early experiments are not included in the curves of difference values. There can be no objection, however, to plotting the data on the curves of absolute values since these represent the actual amount of lactic acid in the tissues. The rate at which lactic acid is formed in the various tissues might be read from both sets of curves but as the curves plotted from difference values all start from the zero line they afford a better comparative representation of the rate of lactic acid formation. The curves of absolute values are drawn primarily to show the lactic acid content of the different tissues at any given moment after the onset of asphyxiation.

At the outset of the experiments it was assumed that in a group of animals selected at random there would occur such marked variations in the lactic acid content of a given organ that time curves showing the rate of lactic acid formation would need to be constructed from difference values obtained by taking the difference between the lactic acid content of an organ frozen immediately after decapitation and that of the symmetrical organ incubated for a specified period of time. McGinty and Gesell (1925) by this method succeeded in constructing a curve showing the rate of lactic acid metabolism in the brain. In the course of the pres-

ent experiments it became apparent that curves could be constructed by plotting on the abscissa the absolute amounts of lactic acid in the brain, kidney and muscle per 100 grams of tissue at various intervals after decapitation, against time on the ordinate (figs. 1a, 2a, and 3a). The lactic acid in the testicles of young dogs on the other hand varies within such wide limits that it is impossible to construct a curve from these values (fig. 4a). Since this organ is presumably grossly affected in its functional development by the age of the animal, the variations might probably be due to age differences.

Although some of the points in figures 1a, 2a, and 3a lie off the curves these data point to a fairly constant and characteristic amount of lactic acid in brain, kidney and muscle tissue both under normal conditions and at any given moment of anaerobiosis. Normal brain tissue contains approximately two to three times as much lactic acid as kidney and muscle whereas there is but a slight difference between the normal content of the kidney and muscle. Extrapolation of the curves places the normal values at 50 to 70 mgm., 10 to 30 mgm. and 15 to 35 mgm. per cent for the brain, kidney and muscle respectively.

The values obtained in these experiments for the brain and muscle are in fairly close agreement with those reported by other workers. McGinty and Gesell (1925) calculated from their data the average initial lactic acid content of brain tissue to be 75.2 mgm. per cent. This value is perhaps a little too high for the Clausen method of extracting lactic acid was employed which may yield 10.2 mgm. per cent more lactic acid from blood than the Friedemann, Cotonio and Shaffer method. The latter, it appears, is more accurate since Ronzoni and Wallen-Lawrence (1928) found that the lactic acid calculated from the carbon monoxide liberated from lactic acid checks with the amount extracted by the Friedmann method. McGinty and Gesell themselves made a few direct determinations by precipitating lactic acid as zinc lactate from which they concluded that their results with the Clausen method were somewhat high for lactic acid.

Since the memorable studies of Fletcher and Hopkins (1907) in which lactic acid values as low as 20 mgm. per cent were reported for frog muscle, a number of workers have obtained similarly low values for resting mammalian muscle. Davenport and Davenport (1927) found that resting atonic muscle frozen in situ of the guinea pig anesthetized with amytal, contained 10 to 20 mgm. per 100 grams of tissue. Simpson and Macleod (1928) reported that in a series of eight experiments the initial lactic acid content of the gracilis and semitendinosus of the cat varied from 30 to 70 mgm. per cent, and in another experiment was as low as 24 mgm. per cent. Ronzoni (1928) obtained 30 to 60 mgm. per cent lactic acid in striated muscle of the chicken frozen in situ. Gesell, Krueger, Nicholson, Brassfield and Pelecovich found that muscle removed from a dog anesthetized

with morphine and urethane in three experiments had an initial lactic acid content of 30, 41 and 54 mgm. per cent.

Relatively few data have been reported on the lactic acid in the kidney and testicle. Irving (1928) found that in five experiments on the cortex of the rabbit's kidney it varied from 45 to 82 mgm. per cent with an average of 61 mgm. per cent. Himwich and Jacobson (1927) reported an initial content of 29, 67 and 54 mgm. per cent in the dog's kidney. Unfortunately the authors do not state the time required for freezing the organs. Since the tissues were not frozen quickly in liquid air but triturated in chilled alcohol, it is probable that these figures are too high for the normal lactic acid content. The initial values that have been reported for the testicle are about as variable as in the present experiments, ranging from 6 to 37 mgm. per cent in three experiments (Himwich and Jacobson, 1927) 11 to 31 mgm. per cent in three experiments (Gesell, Krueger, Nicholson, Brassfield and Pelecovich) and 18 to 51 mgm. per cent in four experiments (Himwich and Adams, 1929).

Comparing the curves of absolute values with those of difference values we note that the curves in figures 1 and 2 are very similar to those in figures 1a and 2a respectively. The points in figures 1a and 2a are more scattered than in figures 1 and 2, but this is what might be expected in view of the fact that they represent more data and also the absolute amount of lactic acid in the tissues of different animals. The curves in figures 3 and 3a although similar have a slightly different contour. The straight line in figure 3 may be due to the limited amount of data from which it is constructed. Four or five points several millimeters higher on the ordinate during the first five minutes would give a curve corresponding to the one in figure 3a. The slightly greater rate of increase shown in the curve of absolute values is probably more nearly accurate than figure 3a. As stated above, a curve can not be constructed from the widely scattered values of lactic acid in the testicle. If, however, we plot the values obtained by taking the difference between the lactic acid content of an incubated testicle and the symmetrical organ frozen shortly after decapitation we obtain the curve in figure 4.

Normal lactic acid metabolism is highest in the brain, lowest in the testicle and occupies an intermediate position in the kidney and muscle. In the first five minutes lactic acid in the brain increases nearly 60 mgm. per cent whereas in the muscle, kidney and testicle it increases 20, 25 and 10 mgm. per cent respectively. In these experiments both the oxygen and blood supply are cut off, consequently lactic acid can not be oxidized to any appreciable extent nor diffuse out of the tissues. Experiments of this type in which the factors of oxidation and diffusion are eliminated, we believe, afford an index of what takes place in the tissues under asphyxiation. It would also appear from the above experiments that lactic acid

DIFFERENCE VALUES

Figs. 1–4

ABSOLUTE VALUES
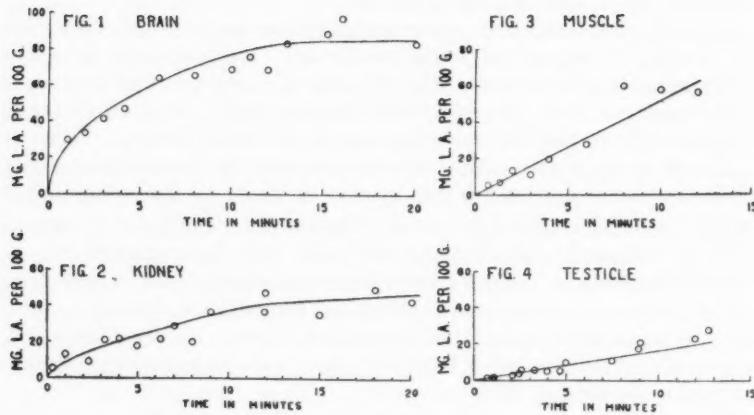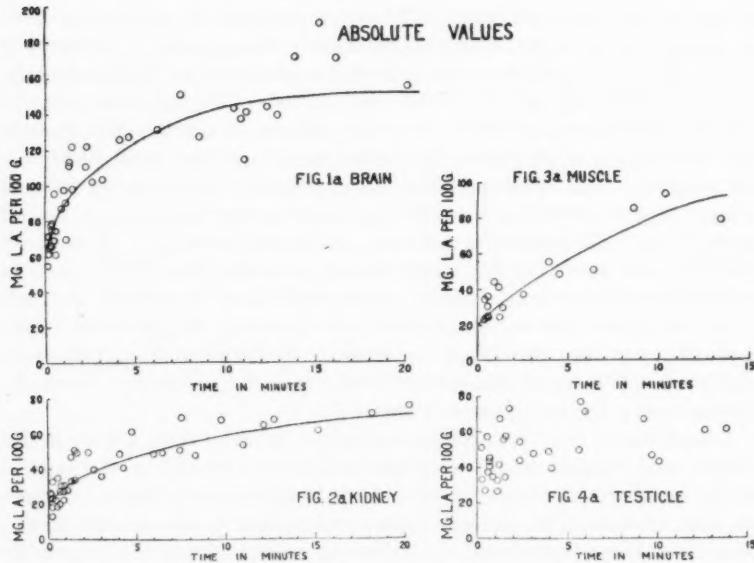
Figs. 1a–4a

is being continually formed in normal tissues and through the processes of oxidation, diffusion or resynthesis into the precursor state, is maintained at a constant characteristic level in each of the tissues.　　In the experiments

of McGinty and Gesell (1925) in which anoxemia was induced by carbon monoxide poisoning, the initial content of lactic acid in the brain was approximately 120 mgm. per cent as against 70 mgm. per cent in normal tissue. These results indicate that in normal tissue an oxidative mechanism is a factor in maintaining the lactic acid content of the tissues at a constant level.

Lactic acid production in the brain proceeds much more slowly after the first five minutes and reaches a maximum in twenty minutes. The curve in figure 1 is essentially in agreement with that reported by McGinty and Gesell. The slight discrepancy in the two curves might be accounted for by the higher yield of the Clausen method. Holmes and Holmes (1925) on the other hand found no increase in the lactic acid of the rabbit's brain after decapitation. These results are undoubtedly due to the length of time (six minutes) that elapsed between decapitation and freezing of the first half of the brain. Since lactic acid forms so rapidly, almost reaching a maximum within ten minutes, the importance of rapidity in removal and freezing of the organ in experiments of this kind can not be over-emphasized.

In the kidney, lactic acid forms most rapidly in the first five minutes although not to as large an extent as in the brain. The second five minutes it continues to increase at a somewhat slower rate. At twenty minutes the curve has not flattened out but is rising very slowly, indicating that lactic acid continues to increase in the kidney beyond twenty minutes. Assuming a continuous ascent of the curve, extrapolation would bring the final values in the neighborhood of the higher figures reported by other workers. Irving (1928) in five experiments on the cortex of the rabbit's kidney obtained an average increase of 86 mgm. per cent in four hours. Himwich and Jacobson (1927) found upon incubating the dog's kidney at 37.5°C. for 3 to 10 hours the lactic acid rose 131 and 266 mgm. per cent.

The curve of lactic acid formation in muscle (fig. 3) is markedly different from the curves for the brain and kidney (figs. 1 and 2). The slope of the curve for the first five minutes is not as steep as the curve in figure 1. At fifteen minutes, unlike the curves in figures 1 and 2, the curve in figure 3 continues as a straight line. Fletcher and Hopkins (1907) likewise obtained a straight line curve for frog muscle incubated at 21°C. which did not flatten out until seventeen hours. Macleod and Simpson (1926) also report a straight line curve for rabbit's muscle.

Lactic acid metabolism in the testicle, as illustrated in figure 4a, proceeds much more slowly than in any of the other organs. In ten minutes the increase is slightly less than 20 mgm. per cent. At the end of thirteen minutes, however, the curve is still ascending as a straight line and in this respect resembles the curve in figure 3 in contrast to the curves in figures 1 and 2. In view of the observations of Himwich and Jacobson (1927)

it appears probable that lactic acid continues to form in the testicle for several hours. In three experiments in which the testicle was incubated three to ten hours they found an increase in lactic acid of 174, 193 and 233 mgm. per cent.

How can we account for the fact that lactic acid forms extremely rapidly in the normal brain, very slowly in the testicle, and, relatively speaking, only at a moderate rate in the muscle and kidney? Meyerhof's (1926) extraction of an enzyme from muscle, hydrolyzing glycogen to lactic acid, suggests a hypothetical explanation. It is not improbable that the other tissues contain a similar enzyme. Assuming that this is the case, should the enzyme be present in a higher concentration in the brain and in a lower concentration in the testicle than in the muscle, the differences in the rate of lactic acid formation might thus be accounted for on the principle that the rate of an enzyme controlled reaction is proportional to the concentration of the enzyme. There is also the possibility that other enzymes may be found in the tissues oxidizing lactic acid or resynthesizing lactic acid to precursor. If such enzymes are present in the tissues the rate of lactic acid metabolism would be determined by their relative concentration.

SUMMARY

Comparative determinations were made of the normal lactic acid content of the brain, kidney, muscle and testicle of the dog.

The rate of lactic acid metabolism in these organs under complete asphyxiation was also determined.

In view of the variations that might be expected in tissues of different animals the lactic acid content of the normal brain, kidney and muscle was remarkably constant. The brain contained 50 to 70 mgm., the kidney 10 to 30 mgm. and muscle 15 to 35 mgm. lactic acid per 100 grams of tissue.

The lactic acid content of the normal testicle on the other hand was extremely variable. This variability might possibly be attributed to age differences.

Under anaerobic conditions there was a marked difference in the rate of lactic acid formation in the various tissues. The rate of increase was most rapid in the brain, slowest in the testicle and intermediate in the kidney and muscle. The initial content of the brain rose approximately 80 mgm. per cent in ten minutes whereas in the kidney, muscle and testicle the increase was approximately 35, 50 and 15 mgm. per cent respectively.

It is suggested that the differences in the rate of lactic acid formation in the various tissues might be due to different concentrations of enzymes controlling lactic acid metabolism.

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THE EFFECT OF THE ADRENAL CORTICAL HORMONE UPON THE RESPIRATORY METABOLISM OF THE CAT[1,2]

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There has been little doubt in the minds of many investigators, for a long time, that the adrenal cortex plays a rôle in the regulation of respiratory metabolism in the body. The nature of this rôle has been difficult to determine. Attempts to do this have largely been along two lines. One group of workers have practised complete removal of the adrenal glands in animals and studied the respiratory metabolism until death occurred in these animals. The second group have attempted sublethal injury of the adrenal cortex, thus allowing a longer time period over which to study the respiratory exchange. Because of these two methods of attack and the diverging results obtained, some confusion exists in the literature on the subject. With the introduction of an active extract of the adrenal cortex by two of the writers (1), a new means of studying the effect of the adrenal cortex upon the respiratory metabolism became available. The material presented in the following experiments represents an attempt to approach the problem along that line.

Golyakowski in 1899 (2) published a report of his observations on the metabolism of dogs after almost complete ligation of the blood supply to the adrenal. He reported an increase of 30 per cent in the heat production of those animals which survived for six weeks or longer. Marine and his associates (3) studied the changes in respiratory metabolism in a large group of rabbits after both bilateral adrenalectomy and bilateral destruction of the cortex by freezing with ethyl chloride. They obtained an average increase of 23 per cent in heat production in 57 per cent of their animals. In 9 per cent there was a fall of 26 per cent and in the remainder no change occurred. Scott (4) repeated Marine's experiment using cats

instead of rabbits and concluded that severe but non-fatal injury to the adrenals causes an increase in heat production while more severe injury causes a decrease.   The degree of variation reported was not great.

Gradinescu (5) studied the metabolism of one dog and three cats for from 36 to 53 hours after operation and reported a marked drop.   The details of the method used were not described.

Aub, Forman and Bright (6), in a carefully controlled experiment, determined the metabolic rate of three totally adrenalectomized cats.   They obtained a slight rise in metabolism immediately after operation.   This was followed within 48 hours by a fall to about 25 per cent below normal. This level was maintained until the animals died or were sacrificed, about 5 days after operation.   By means of control experiments they were able to show that the fall in metabolism was not due to decreased food intake or to a fall in body temperature.

Aub (7) in 1922 injected extracts of the adrenal cortex into adrenalectomized animals and failed to obtain any effect on the total metabolism. The exact method of preparation of these extracts was not stated.   Marine, Baumann and Cipra (8) fed a glycerol emulsion of fresh ox-adrenal to adrenalectomized rabbits and obtained a decrease in metabolism in 11 of a series of 18 animals.

The results obtained by these various investigators would appear, at first sight, to be highly conflicting.   There are, however, a number of factors which tend to explain these results.   Marine and his co-workers, (3) (4) supported by Golyakowski, maintain that partial adrenalectomy in the cat or total adrenalectomy in the rabbit is followed by an increase in metabolism.   The explanation for this apparent species difference probably lies in the fact that practically all rabbits have accessory adrenal glands and that removal of the two main adrenals is, in reality, only a partial adrenalectomy.   The majority of reports, in the cases of complete adrenalectomy, seem to indicate that it is followed by a decrease in total metabolism.

EXPERIMENTAL.   The respiratory exchange was measured by Marine's modification of Haldane's open circuit apparatus (9).   Two-hour time periods were used and the accuracy of the apparatus was tested from time to time by blank determinations.   The animals were kept under standard laboratory conditions and were subjected to a period of training at the beginning of the experiment until a constant level of metabolism was reached and maintained for several days.   They were given a constant diet of 100 grams of raw beef liver and 25 cc. of milk daily and were fed approximately 20 hours before the beginning of the metabolism determination.

The adrenal cortical hormone used was prepared by the method previously described by two of the present authors (10).   The adrenalin con-

tent of this extract was between one part in 1,500,000 and one part in 2,000,000.   Injections of the cortical hormone were made subcutaneously, at least 15 hours prior to the beginning of the determination of metabolism.   Owing to the lack of satisfactory methods for the standardization of the adrenal cortical hormone, no attempt was made to quantitate the amount of hormone used against the changes in metabolism.   The method of preparation of the extract was such that 1 cubic centimeter represented 30 grams of fresh beef adrenal cortex.   The actual quantity of hormone present may, however, have varied slightly in different lots of the extract.

It was first considered advisable to determine the effect of the adrenal cortical hormone on the respiratory metabolism of normal animals.   Four adult normal cats were selected and their standard levels of metabolism and body temperature were determined.   The cortical hormone was then injected subcutaneously in quantities of from 6 to 15 cc. daily for periods varying from two to ten days.   After an interval of approximately one week, the experiment was repeated on the same animals, using a different lot of extract.   In all, 12 such experiments were carried out.   In no instance was any appreciable constant variation from the normal level of metabolism observed.   The general health of the cats remained good. Their appetite was unaffected.   There was no change in body temperature. Similar experiments were carried out in three normal adult rabbits with the same result.

The respiratory metabolism of a series of 6 adrenalectomized cats was next studied.   The operation of adrenalectomy was performed under ether anesthesia, in two stages, with at least one week intervening between the removal of the right and left glands.   The normal level of metabolism was established prior to operation and determinations were made daily after operation until the termination of the experiment.   Previous observers (3) have shown that removal of one adrenal does not alter the level of metabolism.   Many of the animals in our series showed some disturbance in metabolism within the first 36 hours after operation.   In some instances it was slightly increased and in others decreased.   However, in no case did this disturbance persist for more than 36 to 48 hours. Following the removal of the second adrenal gland, there was no appreciable change either clinically or metabolically for from 4 to 6 days in our series of animals.   From the sixth to the tenth day there was a progressive fall in the metabolism.   This decrease followed closely the increase in severity of the clinical symptoms.   Forty-eight hours before prostration occurred, the metabolism had usually fallen from 20 to 30 per cent of the normal level.   This decrease continued until a level approximately 40 to 50 per cent below normal was reached.   At this time the animal usually showed early signs of prostration.   The maximum drop in metabolism

which was recorded in two cats 6 to 8 hours before death was approximately 50 per cent.

When the animals were showing definite signs of prostration and the metabolism had fallen to a level from 40 to 45 per cent below normal, cortical hormone was administered subcutaneously in quantities varying between 12 and 24 cubic centimeters daily.   There was slight variation, in the individual experiments, in the exact stage at which the hormone was begun.   In some instances the clinical symptoms were allowed to become more marked than in others.   Consequently, the level of metabolism varied with the degree of these symptoms.   Following the administration of cortical hormone to these prostrate animals, an increase in metabolism occurred within 24 to 48 hours.   In from 48 to 72 hours after the beginning of the administration of the hormone, the metabolism had risen, in each case, to a level varying between 10 and 18 per cent above the normal previously established for that individual animal.   At this point the administration of hormone was stopped and there was a fall within 24 to 36 hours to the normal level.   This was maintained for a varying time period (several days) during which the cat appeared and acted like a normal animal.   At the end of that time the metabolism began gradually to decrease, signs of adrenal insufficiency developed, and the cycle was repeated.   One cat was observed through three such cycles, one through two, and the remainder through one.   The metabolic and clinical changes were essentially the same in each case.   Protocol 1 illustrates the changes which took place in this series of animals.

Twenty-five cubic centimeters of cortical extract were heated to 75°C. for 5 minutes and then injected subcutaneously, in divided doses, into an adrenalectomized cat at a time when its heat production was at a level approximately 35 per cent below normal.   No elevation of metabolism occurred and there was no relief of the clinical signs of adrenal insufficiency. An extract of beef muscle was prepared in the same manner as the adrenal cortical extract (10) and 50 cc. of this were injected subcutaneously, in divided doses, into an adrenalectomized cat whose metabolism was at a low level.   No effect on the animal's metabolism or clinical condition was noted.

At the termination of the experiment all animals were carefully autopsied.   In only one instance was any residual adrenal cortical tissue found at the site of operation.   No accessory adrenal glands were encountered.

The question next arose as to whether or not the thyroid gland played a part in bringing about the elevation of metabolism which occurred when adrenal cortical hormone was injected into bilaterally adrenalectomized animals.   Marine and Baumann (11) have reported a series of experiments in which they found that removal of the thyroid gland prevented or greatly lessened the increase of heat production which they obtained after partial

PROTOCOL 1

Cat 11. Black, male

DATE	BODY WT.	CO_2 GMS., 2 HR. PERIOD	O_2 GMS., 2 HR. PERIOD	R.Q.	TOTAL CAL., 2 HR. PERIOD	CAL. KGM. HR.	BODY TEMP.	
	grams							
Oct. 15.........								Right adrenal removed
Oct. 29.........	2,010	5.81	4.45	0.95	15.77	3.86	102.0	Animal healthy
Oct. 30.........	1,970	4.33	3.90	0.81	13.10	3.35	101.4	
Nov. 2.........	2,130	4.54	4.47	0.74	14.76	3.47	102.2	
Nov. 4.........	2,151	5.30	3.94	0.98	13.83	3.21	101.4	
Nov. 5.........	2,134	4.20	4.18	0.73	13.81	3.23	101.4	
Nov. 6.........	2,239	5.09	4.03	0.92	13.94	3.11	101.3	
Nov. 11.........								Left adrenal removed
Nov. 12.........	1,997	2.85	2.38	0.87	8.15	2.04	98.3	
Nov. 13.........	2,034	4.35	4.40	0.72	14.46	3.55	98.3	Animal appears normal.
Nov. 14.........	2,070	3.95	3.88	0.74	12.85	3.10	97.2	Eating
Nov. 15.........	2,085	3.60	3.09	0.85	10.48	2.51	97.3	Eating full ration
Nov. 16.........	2,090	3.05	2.87	0.77	9.61	2.30	95.2	
Nov. 17.........	2,086	3.21	3.21	0.73	10.55	2.53	96.3	
Nov. 18.........	2,090	3.50	3.26	0.78	10.87	2.60	96.4	
Nov. 19.........	2,060	3.20	2.70	0.86	9.23	2.24	95.6	
Nov. 20.........	2,052	2.60	2.62	0.72	8.62	2.10	95.4	Refusing food. Weak
Nov. 21.........	2,044	2.59	2.54	0.74	8.42	2.06	95.0	Animal very weak, listless. Extract 12 cc.
Nov. 22.........								Slightly more active. Extract 12 cc.
Nov. 23.........	2,022	2.99	2.57	0.85	8.71	2.14	98.2	Much more active. Ate half ration. Extract 6 cc.
Nov. 24.........	2,000	3.76	3.12	0.88	10.66	2.67	100.4	Ate full ration. Appears normal. Extract 12 cc.
Nov. 25.........	1,989	4.16	3.51	0.87	11.90	3.00	100.3	
Nov. 26.........	1,905	3.70	3.49	0.77	11.66	3.06	100.3	
Nov. 28.........	1,911	3.95	3.87	0.74	11.85	3.09	100.4	Appears normal. Active. Eating full rations
Nov. 29.........	1,867	3.54	3.88	0.70	12.06	3.12	98.0	Refused half of food
Dec. 1.........	1,802	1.66	1.69	0.72	5.50	1.53	94.2	Very weak
Dec. 2.........	1,768	1.70	1.77	0.70	5.80	1.63	94.0	Animal prostrate. Extract 30 cc.
Dec. 3.........	1,720	2.54	2.49	0.74	8.26	2.40	94.0	Slightly improved. Ate 10 grams liver. Extract 18 cc.
Dec. 4.........	1,640	2.89	2.72	0.77	9.10	2.78	98.0	Moderately active. Eating half ration. Extract 20 cc.
Dec. 5.........	1,615	2.87	2.78	0.75	9.23	2.87	99.2	Extract 12 cc.
Dec. 6.........	1,607	3.46	3.21	0.78	10.78	3.36	100.4	Extract 12 cc.

PROTOCOL 1—*Concluded*

DATE	BODY WT.	CO₂ GMS., 2 HR. PERIOD	O₂ GMS., 2 HR. PERIOD	R.Q.	TOTAL CAL., 2 HR. PERIOD	CAL. KGM. HR.	BODY TEMP.	
	grams							
Dec. 7.........	1,582	3.52	3.46	0.74	11.48	3.62	100.6	Active
Dec. 8.........	1,545	3.28	3.22	0.74	10.67	3.45	102.0	
Dec. 9.........	1,561	3.29	2.64	0.90	9.16	2.94	98.3	Eating half ration
Dec. 10........	1,530	1.82	1.84	0.72	6.04	1.97	96.0	Moderately weak
Dec. 11........							93.0	Animal very weak. Sacrificed. Autopsy shows no residual adrenal cortical tissue. No accessories found

destruction of the adrenal cortex in rabbits. However, the rapidity with which the changes in metabolism occurred in our series of animals after the administration of hormone, suggested strongly that these changes were independent of the thyroid. Accordingly, the following experiment was devised to demonstrate this hypothesis. A series of 4 normal cats were trained for metabolic determinations. Under ether anesthesia, total thyroidectomies were then performed on these animals. The parathyroid glands were either left in situ or returned to their original location. A gradual fall in the level of metabolism of these animals occurred until, in from 2 to 3 weeks after operation, it had reached a point between 20 and 25 per cent below normal. The cats gained in weight and showed general symptoms of thyroid insufficiency. At this point, bilateral adrenalectomy was performed in two stages, as previously described. The respiratory exchange was measured daily. As was the case in the animals in which the thyroid was intact (12), the metabolism fell coincident with the development of the symptoms of adrenal insufficiency. When these symptoms became severe, injections of cortical hormone were begun. The metabolism rose promptly to a level slightly above the normal and remained there as long as the cortical hormone was administered. In other words, the changes in the respiratory exchange following bilateral adrenalectomy in cats, were essentially the same, in our series of animals, whether the thyroid gland was intact or totally removed. Protocol 2 is typical of the results obtained in all four animals.

The adrenal cortical hormone was injected subcutaneously into 5 myxedematous cats in quantities varying from 10 to 50 cc. each, over a time period of from 1 to 3 days. These animals had been previously rendered myxedematous in the manner described above and the respiratory metab-

PROTOCOL 2

Cat 10.   Male

DATE	BODY WT.	CO₂ GMS., 2 HR. PERIOD	O₂ GMS., 2 HR. PERIOD	R.Q.	TOTAL CAL., 2 HR. PERIOD	CAL. KGM. PER HR.	BODY TEMP.	
	grams							
April 2............	2,352	3.68	3.07	0.87	10.52	2.23	101.0	Normal healthy animal
April 3............	2,443	3.83	3.41	0.82	11.47	2.34	100.2	
April 4............	2,400	3.71	3.30	0.82	11.15	2.32	100.4	
April 5............	2,410	3.74	3.40	0.80	11.44	2.37	101.2	
April 6............	2,406	—	—	—	—	—	—	Thyroidectomy
April 8............	2,375	3.62	3.33	0.79	11.17	2.34	100.3	
April 10...........	2,435	3.65	3.28	0.81	11.04	2.26	101.0	
April 13...........	2,556	3.00	2.80	0.78	9.35	1.83	100.2	
April 15...........	2,644	3.03	2.93	0.75	9.74	1.84	100.4	
April 17...........	2,661	2.93	2.73	0.78	9.03	1.70	100.3	
April 19...........	2,690	2.98	2.69	0.81	9.03	1.68	100.2	
April 20...........	2,720	—	—	—	—	—	—	Left adrenalectomy
April 21...........	2,770	2.95	2.87	0.75	9.49	1.70	100.3	Appears healthy.
April 22...........	2,776	3.91	3.64	0.78	12.21	2.20	100.4	Wound OK
April 23...........	2,765	3.85	3.72	0.75	12.58	2.27	101.0	
April 24...........	2,802	3.27	3.27	0.73	10.75	2.00	100.3	Healthy. Eating full ration
April 25...........	2,827	—	—	—	—	—	101.0	Right adrenalectomy
April 26...........	2,840	4.04	3.82	0.77	12.72	2.24	100.2	Eating full ration.
April 27...........	2,862	3.48	3.65	0.70	11.86	2.07	98.3	Wounds OK
April 28...........	2,806	3.40	2.90	0.85	9.90	1.76	95.4	Early leg symptoms
April 29...........	2,759	2.40	2.22	0.79	7.40	1.34	96.0	
April 30...........	2,730	2.10	1.68	0.91	5.80	1.06	93.0	Unable to walk. Extract 21 cc.
May 1............	2,705	2.07	1.92	0.78	6.45	1.19	93.0	Slightly improved. Extract 21 cc.
May 2............	2,651	3.04	3.08	0.72	10.11	1.90	94.3	More active. Extract 21 cc.
May 3............	2,574	3.90	3.68	0.77	12.25	2.42	98.2	Appears normal. Extract 21 cc.
May 5............	2,580	3.55	3.23	0.80	10.84	2.11	101.0	No symptoms
May 6............	2,604	3.70	3.43	0.78	11.53	2.20	100.2	
May 7............	2,590	3.69	3.53	0.76	11.71	2.26	100.4	
May 8............	2,542	2.59	2.55	0.74	8.42	1.64	96.2	Shows moderate symptoms
May 9............	2,496	1.78	1.85	0.70	6.09	1.22	93.4	Very weak. Extract 15 cc.
May 10...........	2,453	1.90	1.95	0.71	6.38	1.30	94.6	Very weak. Extract 21 cc.
May 11...........	2,400	2.83	2.78	0.74	9.20	1.92	98.2	Much improved. Extract 21 cc.

PROTOCOL 2—*Concluded*

DATE	BODY WT.	CO₂ GMS., 2 HR. PERIOD	O₂ GMS., 2 HR. PERIOD	R.Q.	TOTAL CAL., 2 HR. PERIOD	CAL. KGM. PER HR.	BODY TEMP.	
	grams							
May 12...........	2,339	2.85	2.88	0.72	9.50	2.03	101.0	Extract 21 cc.
May 13...........	2,328	3.15	3.19	0.72	10.47	2.25	101.2	Appears normal. Extract 21 cc.
May 14...........	2,311	3.48	3.44	0.74	11.32	2.45	101.0	Eating full ration
May 15...........	2,286	3.50	3.55	0.72	11.64	2.55	100.4	
May 16...........	2,263	2.74	2.86	0.70	9.36	2.08	100.2	Shows early leg symptoms
May 17...........	2,295	2.15	2.32	0.70	7.33	1.60	93.0	Moderately weak. Ate half ration
May 18...........	2,280	1.79	1.85	0.70	6.10	1.34	93.0	Very weak
May 19...........	2,264	—	—	—	—	—	—	Animal found dead. Autopsy showed no residual adrenal tissue

olism followed daily until it became stabilized at a level approximately 20 per cent below normal.   Within 24 hours after the injection of the cortical hormone into these animals, there was an increase in the respiratory metabolism of from 15 to 30 per cent in 80 per cent of the experiments. This increased level of metabolism was maintained for from 24 to 48 hours after the administration of hormone was stopped.   In 20 per cent of the experiments, no appreciable change in metabolism occurred after the injection of the cortical hormone.   Further, once an elevation of metabolism had been obtained and the animal allowed to return to the myxedematous level, a second rise could not be obtained until a period of 10 to 14 days had elapsed.   We are unable to adequately account for the variability of this reaction.   Further studies along this line are contemplated.

DISCUSSION.   The experiments outlined above appear to indicate that the adrenal cortical hormone exerts an effect on the mechanism which controls respiratory metabolism.

The experiments in which the cats were thyroidectomized and allowed to become myxedematous prior to adrenalectomy tend to show that the effect brought about by injection of the cortical hormone was due to an action of this hormone on the mechanism which controls respiratory metabolism and not to a secondary effect due to its stimulation of the thyroid gland.   Further, the rate at which the metabolism was restored to a normal level suggests that this action was a direct one.   Lusk (13) and others have repeatedly emphasized the fact that marked metabolic changes

due to variations in the thyroxin content of the body occur slowly, the maximum being reached only after an interval of several days. Our experiments would suggest that the adrenal cortex supplies an independent mechanism for the regulation of metabolic changes and that this mechanism is capable of much more rapid action than that which occurs through the

PROTOCOL 3

Cat 9. Black and white male

DATE	BODY WT.	CO₂ GMS., 2 HR. PERIOD	O₂ GMS., 2 HR. PERIOD	R.Q.	TOTAL CAL., 2 HR. PERIOD	CAL. KGM. HR.	
	grams						
Jan. 12	2,435	3.71	3.67	0.73	12.20	2.50	Healthy normal
Jan. 17	2,450	3.98	3.50	0.83	11.81	2.41	animal
Jan. 18	2,490	4.01	3.64	0.80	12.25	2.46	
Jan. 20	2,500	3.84	3.56	0.78	11.97	2.39	
Jan. 22	2,458	3.60	3.45	0.76	11.52	2.34	
Jan. 24	—	—	—	—	—	—	Thyroidectomy
Jan. 26	2,397	3.63	3.54	0.75	11.68	2.43	
Jan. 28	2,442	3.69	3.57	0.75	11.87	2.43	
Jan. 30	2,500	3.74	3.09	0.88	10.60	2.12	
Feb. 1	2,453	3.14	2.81	0.81	9.50	1.93	
Feb. 4	2,488	3.36	2.56	0.95	8.97	1.80	
Feb. 6	2,630	3.01	2.70	0.81	9.10	1.73	
Feb. 7	—	—	—	—	—	—	Extract 15 cc.
Feb. 8	2,680	3.75	3.29	0.83	11.13	2.08	
Feb. 9	2,692	3.71	3.36	0.80	11.33	2.10	
Feb. 10	2,670	3.21	2.89	0.81	9.72	1.82	
Feb. 11	2,644	3.03	2.93	0.75	9.75	1.84	
Feb. 16	2,680	3.32	3.06	0.79	10.24	1.91	
Feb. 20	2,694	3.27	3.03	0.79	10.10	1.88	
Feb. 20	—	—	—	—	—	—	Extract 20 cc.
Feb. 21	2,682	3.63	3.30	0.80	11.10	2.05	Extract 10 cc.
Feb. 22	2,668	3.83	3.41	0.82	11.47	2.19	
Feb. 23	2,640	3.67	3.43	0.78	11.44	2.15	
Feb. 24	2,666	3.09	2.92	0.77	9.76	1.83	
Feb. 26	2,652	3.12	2.75	0.82	9.35	1.79	
Feb. 28							Experiment terminated

thyroid gland. It is possible that it acts directly on the oxidation-reduction processes which take place in the tissues themselves.

An explanation for the elevation of the rate of metabolism in 80 per cent of the series of myxedematous animals, following the administration of large doses of the adrenal cortical hormone, is not apparent. Such a change may represent an attempt on the part of the adrenal cortical hormone to compensate for the deficiency of thyroxin in these animals.

SUMMARY

An attempt has been made to determine the rôle of the adrenal cortex in the regulation of respiratory metabolism. Following bilateral adrenalectomy in cats, there was a maximum fall of approximately 50 per cent in metabolism. The administration of adrenal cortical hormone to these animals caused the respiratory metabolism to return to normal in from 24 to 48 hours. This change also occurred in animals which had been subjected to total thyroidectomy prior to the beginning of the experiment. Subcutaneous injection of large amounts of the adrenal cortical hormone was not found to affect the respiratory metabolism of normal cats or rabbits. Similar quantities of the hormone, when injected into the thyroidectomized cats, caused an increase in metabolism of from 15 to 30 per cent in 80 per cent of the cases. It would appear that the adrenal cortical hormone exerts an influence, either direct or indirect, upon the mechanism of respiratory metabolism and that this effect can occur independently of the thyroid gland.

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